Special Publication of TARI No.145

Proceedings of the Taiwan - USA Symposium on Technology of Cultivation and Molecular Breeding for Ornamental Crops

Edited by
Ting-Fang Hsieh
Ting-En Dai
Liang-Jong Wang

August 18, 2010
TARI, Taichung, TAIWAN

Proceedings of the Taiwan -USA Symposium on Technology of Cultivation and Molecular Breeding for Ornamental Crops

Special Publication of TARI No.145

Proceedings of the Taiwan -USA Symposium on Technology of Cultivation and Molecular Breeding for Ornamental Crops

Edited by

Ting-Fang Hsieh, Ting-En Dai, Liang-Jong Wang

August 18, 2010

TARI, Taichung, TAIWAN

Preface

Ornamental plants are most commonly grown for the display of their flowers used for garden, landscaping and cut flowers, and rated mainly for their esthetic value and attractive features. Fast expanding in recent years, ornamental industry becomes an important agricultural commodity in Taiwan. Therefore, technologies of cultivation and molecular breeding leading to improve unusual characteristics, including leaves, stem, fruit and scent, and pests and diseases resistance are the research activities of commercially needs and provide new and exciting avenues of study.

The USA—Taiwan Symposium on Technology of Cultivation and Molecular Breeding of Ornamental Crops is held on 18 August 2010, with special interests in areas of cultural practices and marker-assisted breeding techniques. We have the privilege to invite eight distinguished scholars from the United States as well as local institutions. Recent advances, either with conventional methods or biotechnology, to the benefit of crop improvement in the particular research programs are presented. Breeders can now introduce genetic variation (e.g., color and scent) into ornamentals by the application of recombinant DNA technology. New and improved strategies (e.g., chemical or radiation induction) of *in vitro* culture have been commercially implemented for the propagation and breeding of a wide variety of ornamental crops. Moreover, advanced cultivation and the

underlying physiological mechanisms are also discussed for bettering the understanding of the environmental factors (eg., light, water, salinity and temperature) that affect plant growth under stress conditions.

It is an honor for Taiwan Agricultural Research Institute, the major governmental agricultural research organization in Taiwan, to host the symposium. On behalf of TARI, I would like to take this opportunity to thank all the speakers who willing to share their useful research findings and the personnel who have contributed their valuable time to the success of the event. The financial support for the symposium by the Council of Agriculture is also greatly appreciated. I believe that the symposium will facilitate interaction among research scientists, educators, growers and others.

Dah-Jiang Liu

Director General
Taiwan Agricultural Research Institute

August 2010

Taiwan -USA symposium on technology of cultivation and molecular breeding for ornamental crops
台美觀賞作物栽培技術與分子輔助育種研討會

Programme Schedule

Time	Topics	Speaker
8:30-9:10	Registration	
9:10-9:30	Opening Ceremony	Dr. Dah-Jiang Liu 劉大江 所長 (Taiwan Agricultural Research Institute, COA, Executive Yuan)
9:30-10:10	Chairperson: Dr. Dah-Jiang Liu 劉大江 所長 (Director-general, Taiwan Agricultural Research Institute, COA, Executive Yuan)	
	Appealing to your senses: Regulation of plant volatile synthesis for enhanced taste and fragrance	Dr. David G. Clark (University of Florida, USA)
10:10-10:40	Tea break/Group photo	
10:40-11:20	Chairperson: Dr. Fur-Chyi Chen 陳福旗 博士 (Professor, National Pingtung University of Science and Technology)	
	Molecular cloning of soybean anthocyanin biosynthetic pathway genes and their applications in flower color engineering	Dr. Chang-Sheng Wang 王強生 博士 (National Chung Hsing University, TAIWAN)
11:20-12:00	Mutation induction of ornamentals *in vitro*	Dr. Chien-Young Chu 朱建鏞 博士 (National Chung Hsing University, TAIWAN)
12:00-13:20	Lunch time	
13:20-14:00	Chairperson: Dr. Ruey-Song Lin 林瑞松 博士 (Professor and Head, Department of Horticulture, National Chung Hsing University)	
	Plant water use and drought stress physiology: Manipulating irrigation for efficient water use and high quality plants	Dr. Marc van Iersel (University of Georgia, USA)

Time	Topics	Speaker
14:00-14:40	Energy-efficient production of greenhouse crops	Dr. Erik Runkle (Michigan State University, USA)
14:40-15:20	Trends of *Phalaenopsis* orchid breeding research	Dr. Fur-Chyi Chen 陳福旗 博士 (National Pingtung University of Science and Technology, TAIWAN)
15:20-15:40	Tea break	
15:40-16:20	Chairperson: Dr. Tsai-Mu Shen 沈再木 博士 (Vice President, National Chia-Yi University)	
	Growth and physiological responses of ornamental plants to salinity	Dr. Genhua Niu (Texas A&M University System. USA)
16:20-17:00	Temperature acclimation before shipping and light acclimation after shipping improved the performance of *Phalaenopsis* after prolonged dark shipping	Dr. Yao-Chien Alex Chang 張耀乾 博士 (National Taiwan University, TAIWAN)
16:20-17:00	Temperature acclimation before shipping and light acclimation after shipping improved the performance of *Phalaenopsis* after prolonged dark shipping	Dr. Yao-Chien Alex Chang 張耀乾 博士 (National Taiwan University, TAIWAN)
17:00-17:30	Discussion	Dr. Dah-Jiang Liu 劉大江 所長 Dr. Fur-Chyi Chen 陳福旗 博士 Dr. Ruey-Song Lin 林瑞松 博士 Dr. Tsai-Mu Shen 沈再木 博士

Contents

Preface i

Presentation schedule iii

Clark D. G. and Colquhoun T. A. - **Appealing to your senses: Regulation of plant volatile synthesis for enhanced taste and fragrance** 1

Wang C. S.- **Molecular cloning of soybean anthocyanin biosynthetic pathway genes and their applications in flower color engineering** 7

Chiang C.Y., Chang H. J., Huang M.C., Huang T. H., Chao Y. J., Fong L. J., Tejakhod S.and Chu C. Y. - **Mutation induction of ornamentals *in vitro*** 23

van Iersel M. and Burnett S. E. - **Plant water use and drought stress physiology: Manipulating irrigation for efficient water use and high quality plants** 31

Runkle E. S. - **Energy-efficient production of greenhouse crops** 55

Chen F. C.and Chin S. W.- **Trends of *Phalaenopsis* orchid breeding research** 75

Niu G.. - **Growth and physiological responses of ornamental plants to salinity** 85

Chang Y. C. A. and Hou J. Y.- **Temperature acclimation before shipping and light acclimation after shipping improved the performance of *Phalaenopsis* after prolonged dark shipping** 105

Appealing to your senses: Regulation of floral volatile synthesis for enhanced fragrance

David G. Clark* **and Thomas A. Colquhoun**

Environmental Horticulture Dept. University of Florida, USA

* Author for correspondence: geranium@ufl.edu

Abstract

At The University of Florida, we now have a critical mass of interdisciplinary researchers focused on delivering new varieties of plants that appeal to the senses of consumers. This unique combination of expertise allows us to 1) understand consumer preferences for different horticultural crops and plant characters and 2) determine the biochemical, physiological and molecular basis of these characters. We utilize information gained in these experiments to guide the development of new varieties of flowering plants, fruits and vegetables – a process we refer to as 'Consumer-Assisted Selection'. At the present time, our main focus is directed toward making flowers that smell better for the next generation of consumers of floriculture crops.

Introduction

The University of Florida Floriculture Biotechnology and Genetics Laboratory was established in 1995 with the central focus of bridging the gap between basic and applied science in the field of floriculture. Using molecular biology tools and genetic engineering, we have determined the function of several genes with potential

applications in the development of new floriculture cultivars. We utilize *Petunia* x *hybrida* 'Mitchell Diploid' (MD) as a physiological and genetic model plant for much of our basic science work. This plant has proven to be very useful because it is a doubled haploid with a short lifecycle, and it is easily transformed using

Agrobacterium tumefaciens, which facilitates phenotyping the effects of transgenes. We also have a large expressed sequence tag (EST) collection made from several different cDNA libraries of different petunia tissues, which facilitates the discovery of new genes with unknown functions that might be useful for making novel plants. MD flowers are also easy to work with because of their large size, and because they emit high levels of volatile organic compounds. These emitted volatiles give 'MD' a characteristic aroma, which is particularly fragrant at night.

Regulation of floral fragrance synthesis

Floral fragrance in petunia is mainly composed of volatile benzenoids/ phenylpropanoids (FVBPs) derived from the shikimate pathway in the plastid. Utilizing our EST database, we have isolated and characterized several FVBP genes that encode proteins which catalyze reactions directly responsible for production of these emitted volatiles. By reducing their expression in transgenic plants using RNA interference (RNAi), we have determined the function of several FVBP genes through observation of loss of specific volatile organic compounds. This has allowed us to construct a putative biochemical model for FVBP synthesis in petunia (Fig. 1).

FVBP genes are coordinately regulated at the level of transcription in various ways. Analysis of spatial expression patterns of all known FVBP biosynthetic pathway genes has shown us that all genes are highly expressed in the petal limb tissues of the corolla, the known site of volatile emission (Underwood et al., 2005;

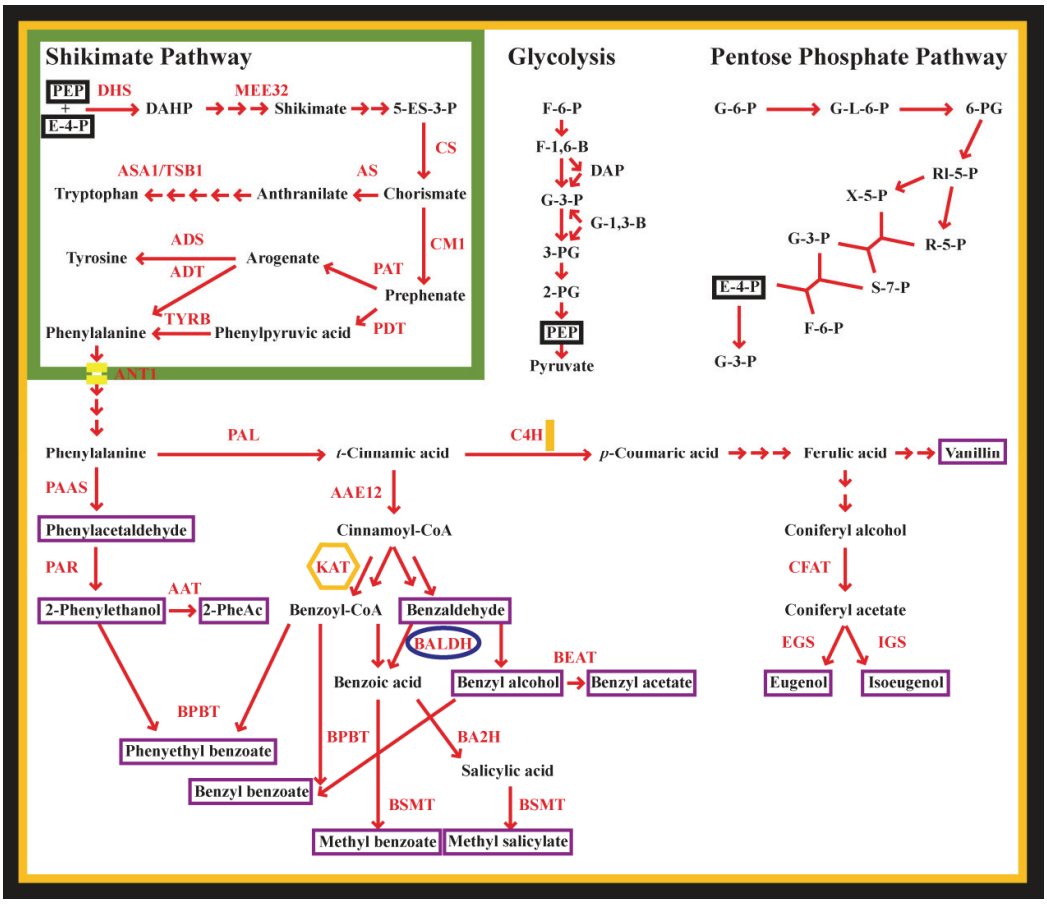


Fig. 1. A biochemical model for synthesis of floral volatile benzenoids/ phenylpropanoids in *Petunia* x *hybrida* 'Mitchell Diploid'.

Colquhoun et al., 2010a). Temporal transcript analysis has revealed that FVBP transcript begin to accumulate in the corolla at the start of anthesis when the flower begins to open. At this developmental stage, the flower becomes receptive to pollination and also capable of emitting fragrance. It is also at anthesis when the corolla becomes sensitive to ethylene which ultimately leads to corolla senescence. Finally, FVBP transcripts are also regulated by an endogenous circadian/diurnal rhythm. Maximum mRNA accumulation of FVBP genes occurs in the mid-late afternoon, which preceeds maximum emission of volatiles around midnight (Colquhoun et al., 2010a).

After spending several years cloning and characterizing genes encoding enzymes responsible for direct synthesis of volatile compounds, we decided to go 'upstream' in the shikimate pathway to determine if similar levels of regulation occur. These efforts led to the isolation and characterization of two genes encoding chorismate mutase (*PhCM1* and *PhCM2* respectively) (Colquhoun et al., 2010b). One of the genes, *PhCM2*, is not regulated in a manner similar to previously isolated FVBP genes. It is expressed in all plant tissues and it is not temporally, developmentally or hormonally regulated. Conversely, *PhCM1* is transcriptionally regulated in a similar manner to previously described FVBP genes. Knock-down (RNAi) *PhCM1* transgenic plants show great reductions in some volatiles such as methyl benzoate and phenylacetaldehyde, but not others such as eugenol and isoeugenol. Thus, *PhCM1* appears to be specific to the process of floral volatile biosynthesis, while *PhCM2* likely has a 'housekeeping' function to maintain phenylalanine synthesis for other cellular processes.

Since we observed that several important FVBP genes share similar patterns of transcriptional regulation, it was logical for us to next search for genes encoding transcription factors that might be responsible for controlling volatile synthesis. Bioinformatic analysis of all known petunia transcription factors identified several candidates of interest. After screening these genes to find which ones are predominantly expressed in flower petal tissues, we were able to isolate several interesting MYB transcription factor genes. Two of these, *PhMYB4* and *PhMYB24* appear to play significant roles in regulating important processes in petunia flowers. Both genes are expressed predominantly in flower petal tissues, and both are developmentally regulated in these tissues in a similar manner to the previously isolated FVBP biosynthetic pathway genes. Using RNAi to knock down expression of *PhMYB4* led to the observed phenotype of a shift in the composition of emitted floral volatiles. Compared to MD plants, *PhMYB4*-RNAi plants produced less methyl

benzoate, but more isoeugenol. Closer investigation showed us that *PhMYB4* acts normally by suppressing the expression of *PhC4H*, which catalyzes the conversion of cinnamic acid to p-coumaric acid. We took a similar approach and made transgenic plants with reduced expression of PhMYB24. Analysis of these plants revealed the striking phenotype of producing flowers that did not open and go through normal anthesis. Apparently PhMYB24 controls expression of several important genes involved in phenylpropanoid production, and also cell wall modification. Thus, this gene is defined as a 'gatekeeper' to the last stages of floral development.

Why is this important?

Our overall goal of this research is to ultimately gain enough information to assist us in breeding new varieties of plants with enhanced fragrance. It is not likely that transgenic plants will make it to US markets any time soon, but we should be able to make gains with new gene markers and analytical techniques. The other part of the equation we are working on is centered on understanding which fragrances humans prefer most. This will be important in making the next generation of fragrant flowers with maximum consumer appeal. We have been utilizing human sensory panels to determine if there are discernable differences between transgenic plants with altered floral volatile synthesis. Not surprising, humans can easily differentiate between normal and engineered plants. In recent work, we have been using facial recognition technologies to quantify human hedonic responses to determine which visual and olfactory characteristics have the greatest influence on human behavior. By analyzing our students at the University of Florida, we feel this approach will be useful in determining which color and fragrance combinations will have the greatest appeal to this next generation of affluent consumers of floral crops. Using a 'Consumer Assisted Selection' approach, we are confident that we can create iconic plants with widespread consumer appeal.

Literature cited

Colquhoun, T. A., Verdonk, J. C., Schimmel, B. C. J, Tieman, D. M., Underwood, B. A., and D. G. Clark 2010a. Petunia floral volatile benzenoid/ phenypropanoid genes are regulated in a similar manner. *Phytochemistry* 71: 158-167.

Colquhoun, T. A., Schimmel, B. C. J, Kim, J. Y., Reinhardt, D., Cline, K., and D.G. Clark 2010b. A petunia CHORISMATE MUTASE specialized for the production of floral volatiles. *The Plant Journal* 61: 145-155.

Clark, D. G., Pichersky, E., Verdonk, J., Dudareva, N., Haring, M., Klahre, U. and R. Schuurink 2009. Benzenoids Dominate the Fragrance of Petunia Flowers. T.Gerats and J. Strommer (eds). Petunia: A monograph. Springer, Dordrecht, Netherlands. p.51-69.

Dexter, R. J., Verdonk, J. C., Underwood, B. A., Shibuya, K., Schmelz, E., Clark, D. G. 2008. Tissue specific PhBPBT expression is differentially regulated in response to endogenous ethylene. *Journal of Experimental Botany* 59: 609-618.

Tieman, D. M., H. M. Loucas, J. Y. Kim, D. G. Clark and H. J. Klee 2007. Tomato phenylacetaldehyde reductases catalyze the last step in the synthesis of the aroma volatile 2-phenylethanol. *Phytochemistry* 68: 2660-2669.

Dexter, R., A. Qualley, C. M. Kish, C. J. Ma, T. Koeduka, D. A. Nagegowda, N. Dudareva, E. Pichersky and D. G. Clark 2007. Characterization of a petunia acetyltransferase involved in the biosynthesis of the floral volatile isoeugenol. *The Plant Journal* 49: 265-275.

Underwood, B. A., D.M. Tieman, *K. Shibuya*, H. M. Loucas, R. J. Dexter, A. J. Simkin, C. A. Sims, E. A. Schmelz, H. J. Klee and D. G. Clark 2005. Ethylene-regulated floral volatile synthesis in petunia corollas. *Plant Physiology* 138: 255-266

Molecular cloning of soybean anthocyanin biosynthetic pathway genes and their applications in flower color engineering

Tsui-Feng Li[1,2], Ren-Mong Liao[1], Chien-Young Chu[2], Junne-Jih Chen[3], Robert Griesbach[4], Chang-Sheng Wang[1*]

[1] Department of Agronomy, National Chung Hsing University, Taiwan (equal contribution)

[2] Department of Horticulture, National Chung Hsing University, Taiwan

[3] Taiwan Agricultural Research Institute, Council of Agriculture, Taiwan

[4] Floral and Nursery Plants Research Unit, USDA, ARS, Beltsville, MD, USA

* Author for correspondence: wangchansen@nchu.edu.tw

Abstract

Color is an important trait for many beautiful flower species which are lack of specific colors due to germplasm limitation. This Taiwan-US collaborative project was initiated to overcome the genetic barrier in breeding new color for several important ornamental crops including pigment gene cloning, vector construction, genetic transformation, and mass propagation of transgenic plants. First, a total of 30 anthocyanin related genes of CRWD soybean variety with *iRT* genotype and a black seedcoats phenotype were cloned for the metabolic engineering in our interest plant species. Second, constructs of cloned genes for overexpression and gene knockout were conducted. Third, agrobacterium mediated transformation systems have been established in chrysanthemum, poinsettia, phalaenopsis orchid to obtain new color.

Cloning and characterization of the cloned genes suggests that the black seedcoats soybean variety contains most complicated anthocyanin biosynthetic pathway and provides the best genetic resource for the metabolic engineering of this pathway. We have been success in engineering flower color by transformation of soybean pigment genes to the above species and confirmed by phenotype, HPLC and/or Southern hybridization.

Introduction

There are always desires for diversities of flower colors from the point of view of consumer therefore who can create new varieties in a short time would have strong competent in market (Mol *et al*., 1995). From all prospects, the genetic transformation will fit this acquirement properly (Tanaka et al., 1998). Flower colors are decided by many factors and among them pigments of anthocyanin is the major group (Inagaki *et al.*, 1999). Anthocyanin pathway is one of unique and well-studied pathway of plant however most studies were focused in the few model plants like maize, snapdragon and petunia (Mol *et al*., 1989; Oud *et al*., 1995; van Houwelingen *et al*., 1998). To our knowledge, the black seedcoat soybean variety must contain homozygous *iRT* genotype and produce most complicated anthocyanin pigments including pelargonidin (sub-pathway 1), cyanide(sub-pathway 2), and delphinidin(sub-pathway 3) since early stage of seed development providing the best material for studying this pathway (Wang et al. 1994). Due to genetic limitation the rose plants contain pelargonidin and cyanide but lack of sub-pathway 3, therefore, no blue rose is available in nature. The sub-pathway 1 and 2 are presented in the pericarps of maize germplasm, tansformation of a maize *DFR* to the red petunia produced pelargonodin and showed brick-red color which was the world first successful flower color engineering and demonstrated a new trend for developing new flower color through molecular breeding (Mol *et al*., 1989).

Strategy for mloecular breeding of flower color

Traditional breeding methods including intra- and inter- specific hybridization and selection have produced many varieties with novel flower color. Moreover, spontaneous or mutagenesis derived mutants are also good population for breeding of novel flower color. However, the limited gene pool in single species may result in restriction of some flower color. For instance, many flowers such as rose and lily have the beautiful flower but without bluish color due to the lacking of flavonoid 3', 5'-hydroxylase (F3', 5'H) enzyme activity for delphinidin accumulation. The cDNA of F3', 5'H cloned from *Vinca major*, *Campanula medium*, *Eustoma russellianum* were overexpressed in petunia and tobacco, both the transgenic plants accumulated delphinidin and light bluish petals were observed (Mori et al., 2004; Okinaka et al., 2003; Shimada et al., 1999). Moreover, *Torenia hybrida* Summerwaves developed by Suntory Ltd., transformed with F3', 5'H has blue flowers (Suzuki et al., 2000). Recently, bluish roses were generated by introduction of viola *F3', 5'H* gene with iris *DFR* gene and down-regulated endogenous DFR expression simultaneously (Katsumoto et al., 2007). These results showed molecular breeding is a powerful method that provides an opportunity to introducing foreign gene to ornamental crops to creates new flower color.

Several steps are required for the genetic engineering of flower cooler：

1. Choose determine a plant species and/or material producing most abundant anthocyanin pigments. Here we choose the soybean variety with black seedcoats as resource for cloning pigment genes because its pathway has been genetically and biochemically studied.
2. Determine the genetic constrain or defect in the target plant species for producing the aimed-color.
3. Clone and construct the gene necessary for providing new flower color to the target

species or variety through genetic transformation.

4. Transform the gene-construct and regenerate the transgenic plants.
5. Screening and analyzing to obtain the transgenic plant with expected phenotype and genotype.
6. The release a new color variety of target species.

Researches on the metabolic engineering of flower color previously at the Laboratory of Molecular Genetics, TARI, and recently at the Laboratory of Genetics and Genomics, Department of Agronomy, NVHU will be reported.

The black seedcoat soybean is a good resource for pigment genes cloning

Flavonoids and isoflavonoids are plants specific secondary metabolites. These C6-C3-C6 compounds are derived from the flavonoid biosynthetic pathway and play important roles in pigment production, pathogen defense, UV protection, hormone biosynthesis, signal transduction, especially for plant to survive from environmental and ecological stresses. Therefore, study on the anthocyanin biosunthetic pathway producing theses secondary metabolites will do great benefit to human being.

In order to conduct metabolic engineering on the anthocyanin biosynthetic pathway soybean CRWD (Ching-Ren-Wo-Dow) variety developed by C. S. Wang with the homozygous *iRT* genotype and black seedcoat phenotype was choosen as the basic material for RNA extraction, cDNA library construction and anthocyanin biosynthetic related genes cloning. So far, genes from ten groups of this pathway have been cloned by cDNA library screening including: 2 CHS (Chalcone synthase), 3 CHI (Chalcone isomerase), 5 F3H (Flavanone 3-hydroxylase), 3 F3'H (Flavonoid 3'-hydroxylase), 1 F3',5'H (Flavonoid 3',5'-hydroxylase) also called blue gene, 7 DFR (Dihydroflavonol 4-reductase), 2 GST（Glutathione S-transferase,）, three ANS

(anthocyanin synthase), 1 FLS (flavone synthase), 1 UFGT, 1 myb, and 1 myc。The molecular characterization of these genes confirmed and suggested that black seedcoat of soybean provide the most complicated anthocyanin biosynthetic pathway as we known. (Fig. 1)

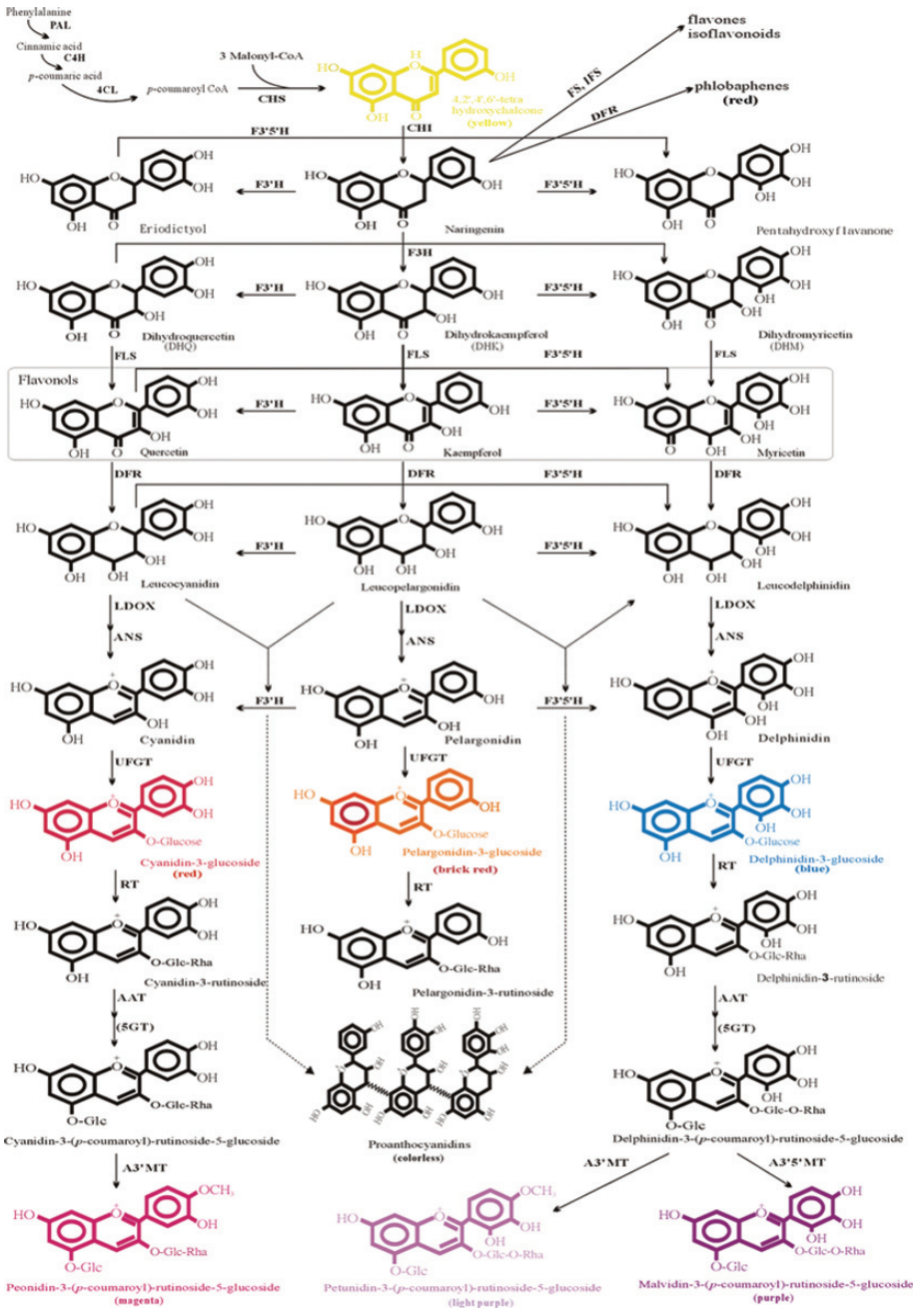
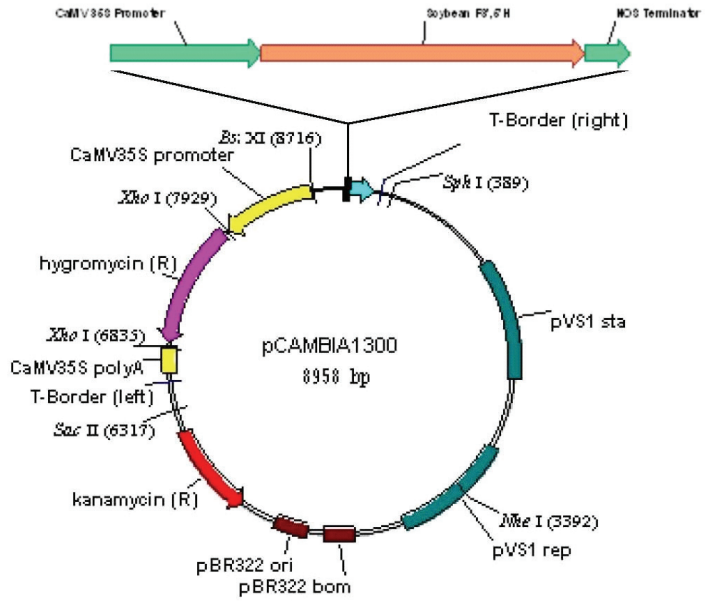


Fig. 1. The anthocyanin biosynthetic pathway in the black seedcoats of soybean.

Construction of transformation vector

In this study, series of pCAMBIA vectors were applied as the backbone for construction of pigment genes (*CHI*, *F3H*, *DFR*, *F3',5'H*) cloned from *Glycine max* 'CRWD' variety for agrobacterium-mediated transformation. For example, F3'5'H driven by 35S promoter (CaMV, cauliflower mosaic virus) was constructed in pCAMBIA1300 as shown in Fig. 2 for transformation. The EHA105 strain of *Agrobacterium tumefaciens* carrying the specific construct was used for transformation.



CaMV 35S-F3,5H-NOS-pCambia 1300

Fig.2. Binary vector used to generate GmF3', 5'H overexpressing tobacco plants.

Establish of transformation system for flower color engineering

In this study, chrysanthemum, phalaenopsis, poinsettia as well as model plant tobacco were chosen as targets for flower color engineering. The agrobacterium-mediated transformation system was established for transformation of the constructs to each plant species.

Gene transformation and regeneration in chrysanthemum

Pigment genes were transferred to all the target plant species by *Agrobacterium*-mediated method, putative transgenic plants were screened in the selection media containing proper antibiotics. An efficient transformation system has been established and as demonstrated in the Fig. 3. Though there exits cultivar specific problems in transformation of chrysanthemum our system can be modified for many commercial variety. All the transformation process can be completed within nine months to obtain flowering plants. After antibiotic screening, they were further screened for both GUS and target genes by PCR. The flower color on petals was analyzed by both PCR and HPLC to detect the integration and expression of the target gene since the early stage of flower development (Figs.4, 5). The transformation of soybean F3H to chrysanthemum Pink diary variety enhanced the pigment synthesis and accumulation at all developmental stages resulted in a darker pink and the color lasting longer duration. The results also supported by the increasing of cyaniding amount the HPLC analysis of pigment amount ranged from 2.03 to 5.75 (Table 1) suggested that transformed with soybean F3H gene may significant enhance follower color and last longer in chrysanthemum.

Transformation of soybean F3H to the Yellow Hsiouphoen variety which contains only carotenoid dramatically turn its color to dark pink and producing cyanidin but no carotenoid (Fig. 5). The result suggested that the carotenoid biosynthetic pathway in the petals of transgenic F3H plant has been shut down and replaced by the a anthocyanin biosynthetic pathway. This was supported by HPLC analysis as shown in Table 2.

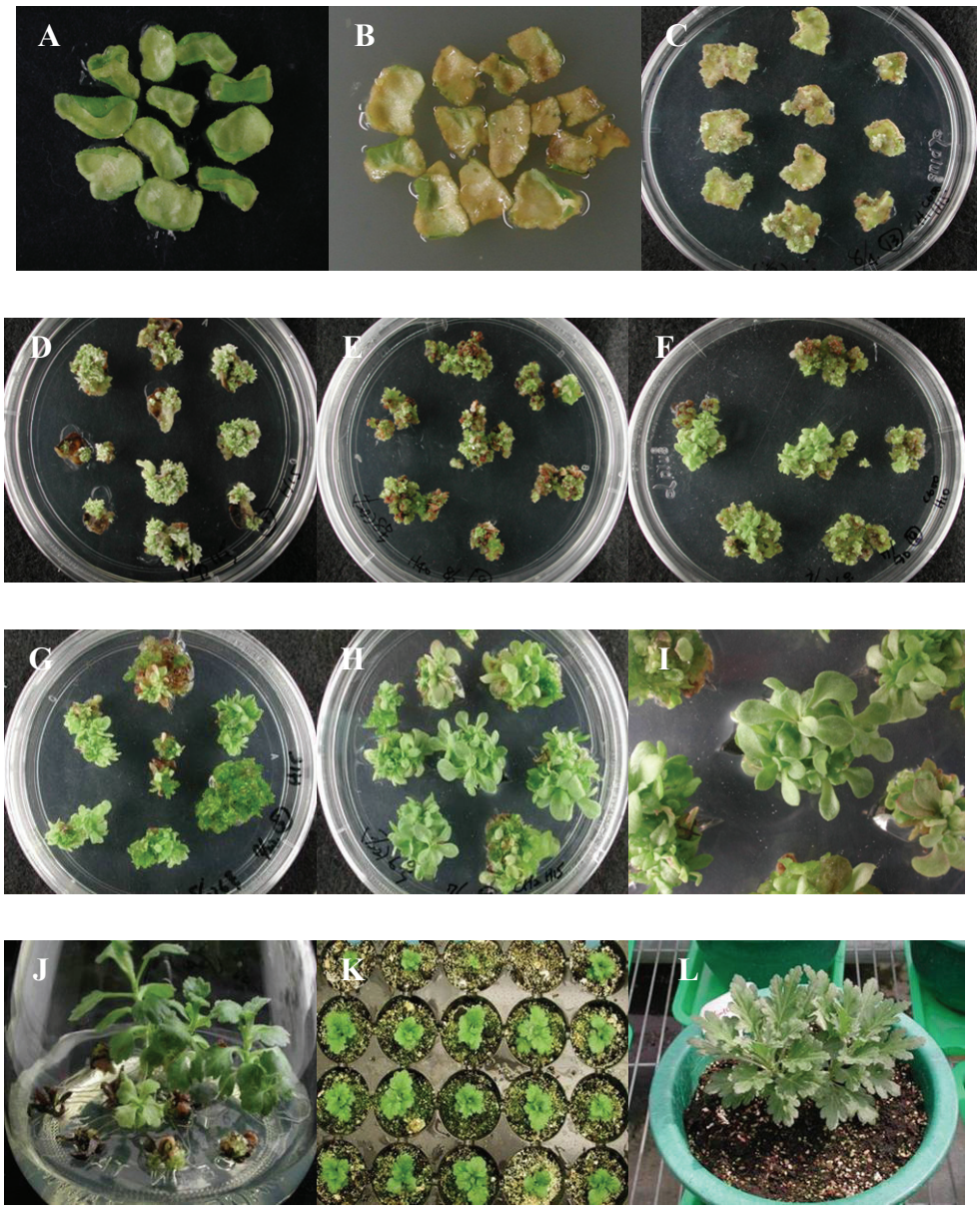


Fig 3. Genetic transformation system for chrysanthemum.
(A) preculture of leaf explant, (B) co-cultivation with agrobacterium, (C) callus formation in selection medium at 1 month after transformation, shoot regeneration at (D) 2 month, (E) 3 month, (F) 4 month, (G) 5 month, and (H) 6 month after transformation, (I)bud elongation, (J) rooting on selection medium, (K) acclimation of PCR screed plants, (L) transgenic plants.

Fig. 4. Pink diary (upper, wild type) transformed with soybean F3H gene (lower) shows enhanced follower color at five stages of flower development.

Table 1. Transgenic Pink Diary produced higher cyaniding amount.

Plant	Flowering Stage（cyanidin, μg/ g FW）				
	1	2	3	4	5
Wild type plant	26.44	46.44	22.77	16.89	10.47
Transgenic plant	82.37	94.3	80.79	62.4	60.16

＊ Not detected

Fig 5. Yellow Hsiouphoen contains carotenoid (upper, wild type), transgenic soybean F3H plant (lower) produces cyanidin and shows pink color.

Table 2. Yellow Hsiouphoen (upper, wild type) transformed with soybean F3H produces cyanidin and shows pink color.

Plant	Flowering Stage（cyanidin, μg/ g FW）		
	1	2	3
Wild type plant	nd*	nd	nd
Transgenic plant	16.7	35.39	11.63

* Not detected

Transformation of pigment genes to poinsettia

A high embryogenesis system has been established for poinsettia in order to introduce new color through genetic transformation (Fig. 6). Two poinsettia varieties, 'Nobel Star' and 'Peter Star-White', were transformed using *Agrobacterium tumefaciens* strain EHA105 carrying soybean pigment genes: F3H, F3',5'H and anthocyanidin synthase (ANS) *via* embryogenesis system. So far, 5 and 7 putative transgenic poinsettia of 'Nobel Star' harboring with F3H or ANS gene, respectively were verified by PCR. Five or 23 putative transgenic poinsettia of 'Peter Star-White' were also verified by PCR to contain F3H or F3',5'H gene, respectively. The phenotypic result will be analyzed in the very near future.

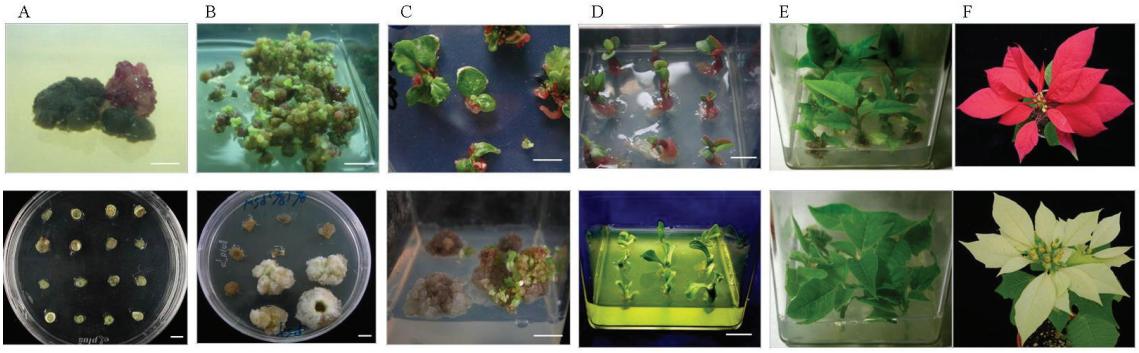


Fig. 6. Process of somatic embryogenesis and transgenic regeneration of poinsettia. 'Nobel Star' (upper) and 'Peter Star-White' (lower). (bar=10 mm)

A. stem explants expansion in the 2nd week (lower), somatic embryos from callus in the 12th week (upper), B. callus induced from stem explant in the 4th week(lower), somatic embryos developed in the 16th week(upper), C. somatic embryos mature and elongate in the 14th week(lower), D. plantlets regenerated from somatic embryos in the 24th week,E. transgenic plants regeneration in the 32nd week, F. plants established and flowering in the greenhouse.

Expression of soybean F3', 5'H gene in tobacco

Tobacco has been a model transformation system for studying gene function. According to our study only sub-pathway 1 is presented in tobacco leading to produces only cyanidin and its derivatives therefore it also serves a good material for studying molecular engineering of flower color. Several F3', 5'H genes were cloned from some plant species, however, only few function were demonstrated in transgenic plants. It was important to investigate the function of *GmF3', 5'H* overexpressed in transgenic plants. Tobacco (*Nicotiana tabacum* cv. Petit Havana) was a good candidate, because it originally lacked F3', 5'H genes and has pink flowers. Binary vector harboring *GmF3', 5'H* driven by the CaMV 35S promoter was transformed into tobacco. In 7 independent transgenic tobacco plants no visible changes other than flower color were observed, apparent changes in flower color intensity were observed in transgenic tobacco plants. The flower color of transformants expressing *GmF3', 5'H* were not blue but magenta and darker than that of control plants. All of them were increased in anthocyanin amount indicated that *GmF3',5'H* hydroxylated B-ring at 3 and 5 positions in transgenic tobacco successfully.

Conclusions

Our results demonstrated that 1. the anthocyanin biosynthetic genes cloned from black seedcoats of soybean can functionally express in many plant species and showed altered metabolites and phenotypes. It's suggested that these genes may provide good resources for metabolic engineering of anthocyanin and its related pathways in plant species. 2. Those unexpected results may due to the complicated and un-identified metabolic network in the target plant species.

Acknowledgements

The authors want to express their sincere thanks to USDA and AIT for their long term financial support on the project “Metabolic engineering of flower color” (91-SA-1.4-IC-01(2)) and travel grant to C. S. Wang. Initiation of this project by Dr. Teh-Yeh Ku and coordinated by Dr. Ming-Jhe Wu are also acknowledged.

Literature cited

Boase, M. R., R. C. Butler, and N. K. Borst. 1998. Chrysanthemum cultivar – *Agrobacterium* interactions revealed by GUS expression time course experiments. Sci. Hort. 77: 89-107.

Chen, Y. M., C. S. Wang, and C. Y. Chu. 2006. Flower bud as explant for Agrobacterium tumefacien-mediated transformation of pigment gene in chrysanthemum. Horticulture NCHU 31: 81-94.

Fukai, S., J. D. Jong, and W. Redemaker. 1995. *Agrobacterium*-mediated Genetic transformation of *chrysanthemum*. Acta Hort. 392: 147-153.

Hooykaas P. J. J. and Schilperoort R. A. 1992. *Agrobacterium* and plant genetic engineering. Plant Mol. Biol. 19: 15-38.

Kudo, S., N. Shibata, Y. Kanno, and M. Suzuki. 2002. Transformation of *chrysanthemum* Via *Agrobacterium tumefaciens*. Acta Hort. 572: 139-147.

Ledger, S. E., S. C. Deroles, and N. K. Given. 1991 Regeneration and *Agrobacterium*-mediated transformation of *chrysanthemum*. Plant Cell Reports 10: 195-199.

Li, T. F., C. S. Wang, and C. Y. Chu. 2008.Transformation of Soybean Pigment Genes

to Poinsettia. Hort. NCHU 31: 81-94.

Lu, C. Y., G. Nugent, and T. Wardley. 1990. Efficent, direct plant regeneration from stem segments of *chrysanthemum*. Plant Cell Reports 8: 733-736.

Mol J. N. M., Stuitje A. R. and van der Krol A. 1989. Genetic manipulation of floral pigmentation genes. Plant Mol. Biol. 13: 287-294.

Mol J. N. M., Holton T. A. and Koes R. 1995. Floriculture: genetic engineering of commercial traits. TIBTECH. 13: 350-355.

Sanford J. C., Smith F. D., and Russell J. A. 1993. Optimizing the biolistic process for different biological applications. Meth. Enzymol. 217: 483-509

Sherman, J. M., J. W. Moyer, and M. E. Daub. 1998. A Regeneration and *Agrobacterium*-mediated transformation System for Genetically Diverse *chrysanthemum* Cultivars. J. Amer. Soc. Hort. Sci. 123(2): 189-194.

Smith R. H. and Hood E. E. 1995. *Agrobacterium tumefaciens* transformation of monocotyledons. Crop Sci.35: 301-309.

Tanaka Y., Tsuda S. and Kusumi T. 1998. Metabolic engineering to modified flower color. Plant Cell Physiol. 39: 1119-1126.

Wang C. S and Vodkin LO (1994) Extraction of RNA from tissues containing high levels of procyanidins that bind RNA. *Plant Mol Biol Rep* 12: 132-145.

Wang, C. S., J. J. Todd, and L. O. Vodkin. 1994. Chalcone synthase mRNA and activity are reduced in yellow soybean seed coats with dominant *I* alleles. Plant Physiol. 105: 739-748.

Wang. C. S., C. N. Hsia, J. J. Chen, R. J. Griesbach, and H. T. Hsu. 1997. Developing

new flower colors by transformation of pigment biosynthetic gene(s). Proceeding of the symposium for Reviewing COA-USDA Agricultural Science Cooperative Program, Taichung, Taiwan.

Wang, C. S. 1999. Cloning of anthocyanin biosynthetic genes and molecular genetic studies at TARI. Proceedings Symposium of Scientific Cooperation in Agriculture between INRA (France) and Council of Agriculture (Taiwan, R.O.C.) pp. 285-289. April 19-20, Toulouse, France.

Zheng, Z. L., Z. Yang, J. C. Jang, J. D. Metzger. 2001. Modification of plant architecture in chrysanthemum by ectopic expression of the tobacco phytochrome B1 gene. J Am Soc Hort. Sci. 126: 19– 26.

Mutation induction of ornamentals *in vitro*

C.Y. Chiang, H. J. Chang, M. C. Huang, T. H. Huang, Y. J. Chao,

L. J. Fong, S. Tejakhod, C. Y. Chu*

Departmen of Horticulture, National Chung Hsing University, Taiwan

* Author for correspondence : chychu@dragon.nchu.edu.tw

Abstract

Plant tissue culture is an efficient technique when it comes to plant propagation. The feasibility of combining plant tissue culture with mutagenesis through chemical or physical agent has been tested in our lab. Crops tested include chrysanthemum, *Dieffenbachia*, *Gerbera*, poinsettia and *Kalanchoe*.The higher mutation rates of most chrysanthemums were obtained from callus under γ-ray irradiation at 4 Gy. As well as *Dieffenbachia* was obtained at 1 Gy. The mutation types from compositae flower bud explants had more varieties than those obtained from callus explants, and that replacing γ-ray with NaN_3 as mutagen caused severe decrease in mutagenesis efficiency.The poinsettia hypocotyl explants cut from mature embryos were used and exposed under γ-ray or were subcultured on regenerating medium containing NaN_3. In the end, we found that physical mutagen treatment was more suitable for organgensis and chemical mutagen was more suitable for embryogenesis.lower buds of kalanchoe were exposed to NaN_3, and mutation rates of 77.8 to 53.1% were obtained.

Introduction

Demand for ornamental diversity has always been important in the ornamental market. Creating different flower colors, shapes, while maintaining unified plant type and cultural characteristics contribute to such varieties among ornamental commodities. To this fact, many methods in producing plants with new characteristics have been developed. One of the most commonly used methods is mutagenesis through exposing plants to chemicals (e.g. NaN_3) or radiation (e.g. γ-ray). By treatment of plant cutting under these factors, plant breeders are able to obtain new cultivars far exceeding conventional plant breeding techniques (such as cross breeding… etc). To this date, many ornamental cultivars have already been developed through these techniques and received fruitful results. In spite of its success in plant breeding, however, the high cost in labors, expenses and time of this technique still call for further improvements in its efficiency in propagation and phenotype screening.

Plant tissue culture is an efficient technique when it comes to plant propagation. In addition, it is capable for year-round production and possesses less chimera problems. In view of its advantages, it seems promising that a combination between plant mutagenesis and plant tissue culture may provide a plant breeding technique with greater efficiency and lower cost. The feasibility of combining plant tissue culture with mutagenesis through chemical or physical agent has been tested in our lab. Crops tested include chrysanthemum, *Dieffenbachia*, *Gerbera*, poinsettia and *Kalanchoe*. The following contents address the attempts made and progress received in our lab.

Mutagen on callus culture

Our attempts started off by applying γ-ray irradiation on calli of chrysanthemum and *Dieffenbachia*. Sterilized leaf discs of these crops were cultured in MS agar medium containing with 30 g/L of sucrose, 5 mg/L of IAA, 10 mg/L of BA and 1 mg/L of 2,4-D under pH5.7 to induce callus formation. The culture was conducted under 25±3℃ and 35±5μmolm^{-2}s^{-1} of lightness for 16 hrs photoperiod. Calli were further multiplied in MS medium containing 1 mg/L of BA and 0.5 mg/L of IAA. After 1 week of subculture, the callus explants were exposed under γ-ray radiation (1~4Gy). Following the formation of adventitious shoots and ex vitro of cuttings, plants with extraordinary phenotypes were selected, cloned a grown into cultivars. Results showed that mutation rates of the treated plants differed between different cultivars, with chrysanthemum 'Red Beauty' showing the highest mutation rate. In spite of such differences, the highest mutation rates of most cultivars were obtained under the same dosage of γ-ray irradiation (4Gy).

The same method was also tested on *Dieffenbachia*. In this plant, different mediums was used (1/2MS basal medium containing BA 3 mg/L and NAA 0.25 mg/L, or BA 5 mg/L and NAA 0.125 mg/L, respectively) and the mutation induction was most effective under 1Gy of γ-ray radiation. In the end, many varieties of *Dieffenbachia* were bred successfully.

Mutagen on flower culture

We continued our study on the mutation of spray chrysanthemum and *Gerbera*. Since head flowers (special inflorescence of compositae) are sterilized easier, the efficiency of the combination of flower bud culture with induced mutagenesis in these plants was tested. Our findings indicated that, after mutagenesis induction, efficiency

of developing adventitious bud from spray chrysanthemum and *Gerbera* head flowers differed among different cultivars, with the shoot development stage as the optimal timing for mutagenesis induction, and 4Gy γ-ray irradiation (once or twice) as the optimal dosage. In addition, we found that mutation types from flower bud explants had more varieties than those obtained from callus explants, and that replacing γ-ray with NaN_3 as mutagen caused severe decrease in mutagenesis efficiency.

Mutation via embryogenesis

Another attempt was made on poinsettia according to our previous finding in poinsettia micropropagation that callus explants of poinsettia can proliferate into embryos easily, and the fact that most poinsettia cultivars were developed by mutation. Poinsettia shoot tip were grown on MS medium containing 0.4~0.8 mg/L of 2,4-D and 0.2 mg/L of BA to induce callus, and subcultured on the same medium for callus multiplication. Following the embryogenesis of these calli on MS medium containing NAA 0.2 mg/L and BA 0.2 mg/L, they were exposed under 1~4Gy γ-ray radiation (once or twice). Global embryos formed were used as explants for secondary embryos, which were then, after applying riboflavin in medium, developed into cotyledon embryo. Finally, the hypocotyl explants cut from mature embryos were used and exposed under γ-ray or were subcultured on regenerating medium containing NaN_3. In the end, we found that physical mutagen treatment was more suitable for organgensis and chemical mutagen was more suitable for embryogenesis.

Finally, our most recent study focused on the breeding of *Kalanchoe garambiensis,* an endemic and endangered species in Taiwan, which is known to produce few offspring with low or poor fertility after inter specific crossing. In addition, many species of *Kalanchoe* can also produce plantlets on the leaf margin through embryogenesis. Flower buds of kalanchoe interspecific cultivars ‘Beacon’

and ‘Sunrise’ were exposed to NaN_3, and mutation rates of 77.8 or 53.1% were obtained respectively. Flower colors of these mutants changed from bright orange to oreddish orange, light orange or yellow. Moreover, leaf phenotype changes were also observed in these mutants.

In conclusion, our approach of combining plant mutagenesis with plant tissue culture in ornamental breeding has received fruitful results in chrysanthemum, *Dieffenbachia*, *Gerbera*, poinsettia and *Kalanchoe*. With little modifications in the culture condidiond of these plants, and the correct choice of type and dosage of mutagen, this technique shall provide us an efficient way in ornamental breeding.

Acknowledgements

Thanks to the Council of Science and Council of Agriculture for financial support: NSC 89-2313-B-005-074, NSC 89-2313-B-005-203, NSC 90-2313-B-005-071, NSC 91-2313-B-005-107, NSC 92-2313-B-005-079, NSC 93-2313-B-005-019, 92AS-4.2.3-F-Z3(1), 93AS-4.2.1-F-Z1(2)。

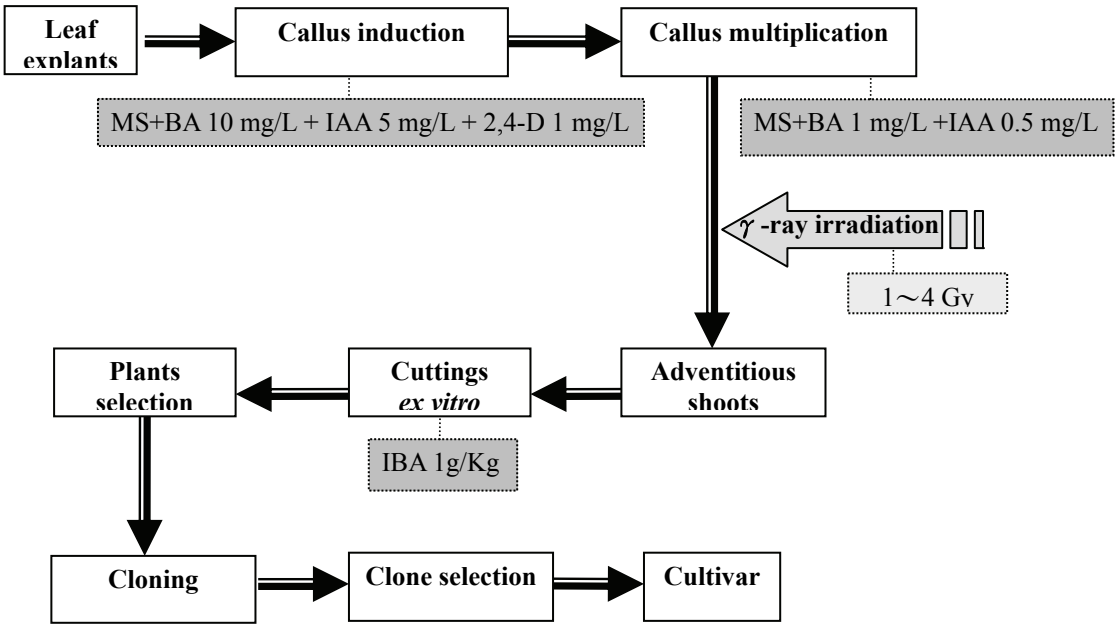


Fig.1. The procedure of chrysanthemum mutagenesis from leaf callus

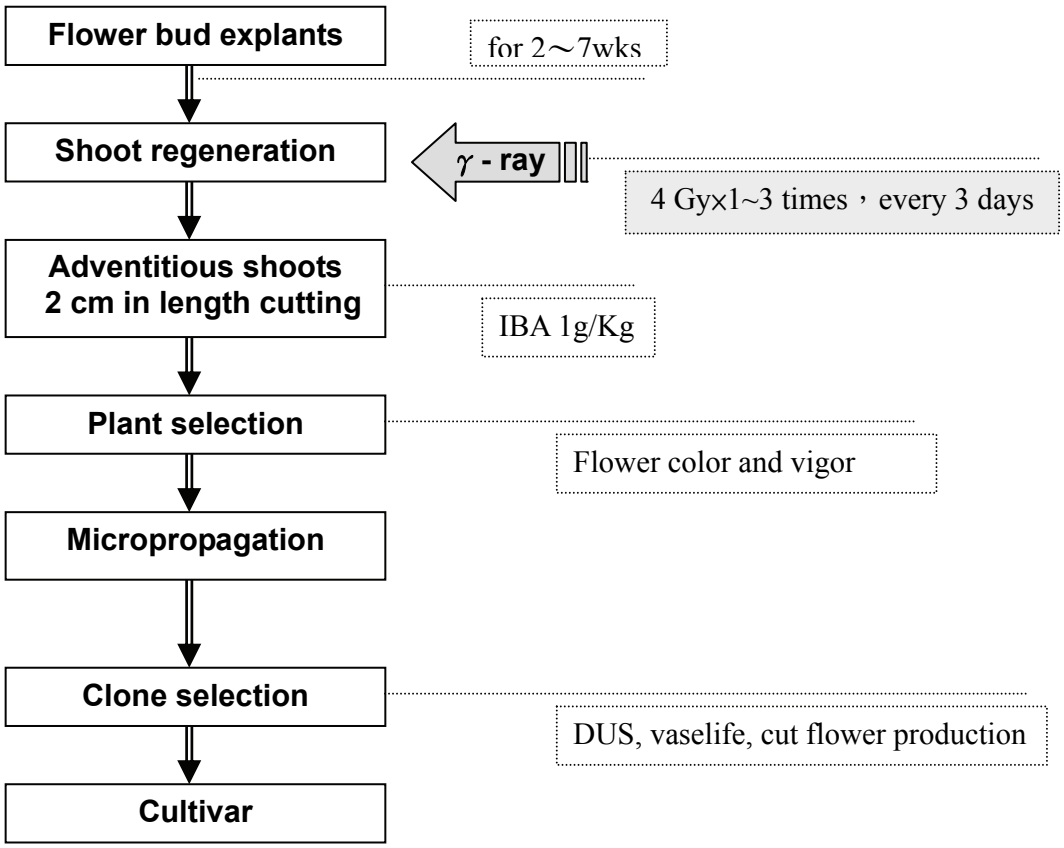


Fig.2. Procedure of chrysanthemum or gerbera mutagenesis from flower buds

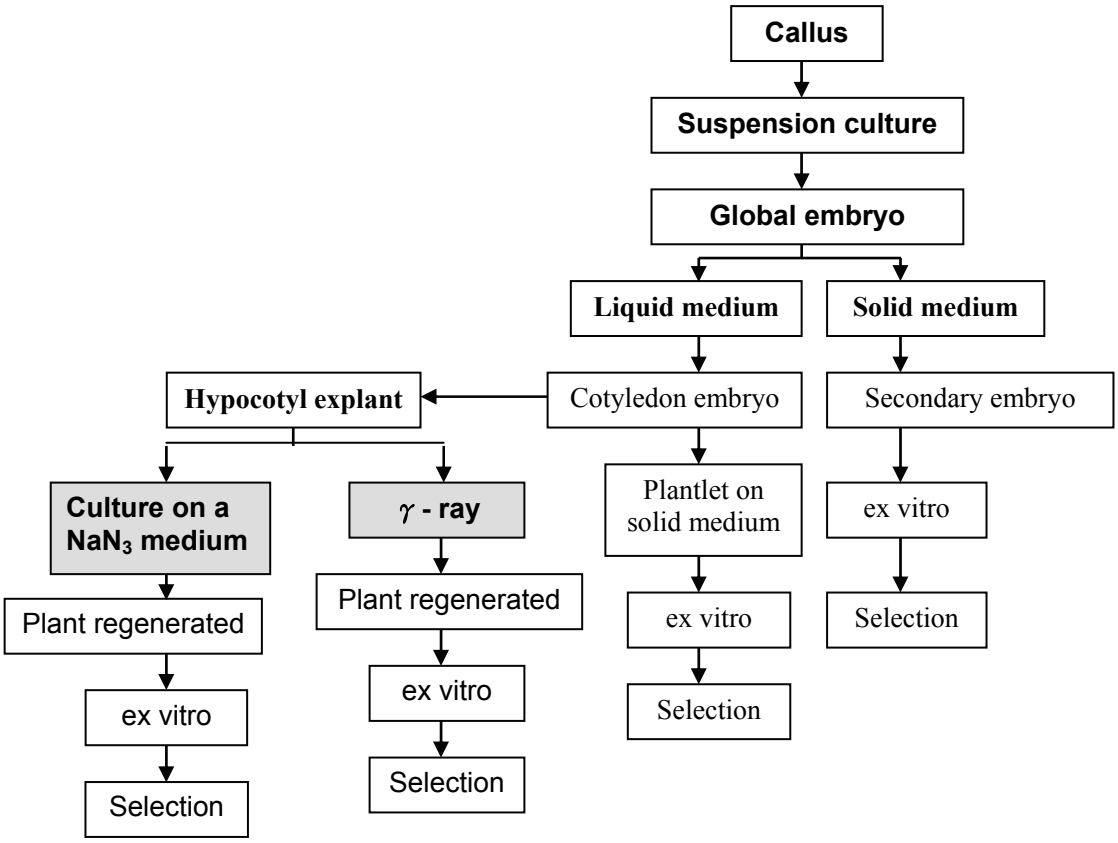


Fig.3. Poinsettia embryogenesis and mutagenesis in liquid medium

Literature cited

Chu, C. Y., M. C. Huang and C. Y. Chiang. 2000. Development of new spray chrysanthemum (Dendranthema × grandiflorum (Ramat) Kitamura) by callus culture irradiated γ-ray. SEED & NURSERY 2(2): 143-156.

Chu, C. Y., C. Y. Chiang and T. H. Huang. 2004. Application of biotechnology on poinsettia breeding(II). Council of Agriculture, 2004 research project report.

Chiang, C. Y. and C. Y. Chu. 2006. Plant regeneration from capitulum, callus and suspension culture of chrysanthemum (*Dendranthema* × *grandiflorum* (Ramat)

Kitamura) after γ-ray irradiation. SEED & NURSERY 8(3): 19-28.

Chang, H. C.and C. Y. Chu. 2005. Gerbera mutation breeding via floral culture with SA。SEED & NURSERY 7(2): 23-33.

Hsiao, Y. Y. and C. Y. Chu. 2002. Micropropagagtion and Mutation of *Dieffenbachia maculata* 'Rudolph Roehrs-86M_1'. Horticulture NCHU 27(1): 41-54.

Plant water use and drought stress physiology: Manipulating irrigation for efficient water use and high quality plants

Marc van Iersel [1]*, Stephanie E. Burnett [2]

[1] Department of Horticulture, University of Georgia, 1111 Miller Plant Science Building, Athens, GA 30602, USA

[2] Department of Plant, Soil, and Environmental Sciences, University of Maine, 5722 Deering Hall, Orono, ME 04469, USA

* Author for correspondence : mvanier@uga.edu

Abstract

Irrigation systems that can control watering based on soil moisture sensors are a powerful tool for both greenhouse irrigation and drought stress physiology research. This paper provides an overview of research at the University of Georgia. Three studies are discussed: a study describing the effects of substrate water content (θ) on growth and water use of petunia, a study looking at growth and drought stress physiology of vinca, and a study looking at control of both irrigation and fertigation using *in situ* soil moisture and electrical conductivity sensors. This research has practical applications for greenhouse irrigation and fertilization management, while also furthering drought stress physiological research.

Introduction

Managing global water resources is one of the most pressing challenges of the 21st century. Population growth and increased urbanization have increased competition for water by agricultural, industrial and domestic users. Agricultural water use in many areas of the world is not sustainable (Jury and Vaux, 2005). To meet the long-term needs of the world's population, it is crucial that the efficiency of agricultural water use is increased (Howell, 2001) and science and technology must play an important role in addressing problems arising from a global water shortage.

The greenhouse industry is not immune from these issues, and improving irrigation efficiency must play an important role in making greenhouse production more sustainable. Various US states have legislation regulating agricultural water use and quality. Some legislation requires greenhouses and nurseries to develop nutrient management plans describing efforts to reduce non-point source pollution (Lea-Cox and Ross, 2001). Sustainable greenhouse irrigation practices are not just important for the US but also in many other greenhouse production areas.

Good irrigation management is an important best management practice in ornamental crop production, reducing runoff of nutrient- and pesticide-rich water from production sites (Tyler et al., 1996; Briggs et al., 1998, Lea-Cox and Ross, 2001). Better control of irrigation can have other benefits as well: better plant quality, more compact plants (Burnett and van Iersel, 2008) and reduced damage from root pathogens (Powell and Lindquist, 1997). In addition, more efficient irrigation can have direct economic benefits, since less electricity or fuel is needed to pump the irrigation water.

A promising approach for improving irrigation practices is the use of real-time sensing technology to detect the water status of the soil or substrate and to use those

measurements to control irrigation. This approach is readily applied and allows for easy automation (Jones, 2004). Jones (2007) concluded that soil water content is the most valuable measure of plant or soil water status for purposes of irrigation scheduling.

We have developed an automated irrigation system that measures θ in multiple containers, and irrigates them based on container-specific θ thresholds. This irrigation system can maintain θ within a narrow range and automatically adjusts irrigation as plants get larger or environmental conditions change (Nemali and van Iersel, 2006). We have since modified this irrigation system to allow for precise determination of the daily amount of water applied to each container. This irrigation system allows for maintenance of different substrate water content (θ) levels, and thus makes it possible to evaluate whole plant physiological and morphological response to drought stress.

An even more recent development is the availability of sensors that can measure both θ as well as the bulk electrical conductivity (EC) of the substrate. Since EC can be used as an indicator of nutrient availability in the substrate, these θ and EC sensors raise the possibility of automating not only irrigation, but also fertilizer applications. Integrated control of irrigation and fertilization is especially important in greenhouses, since water-soluble fertilizer is generally applied together the irrigation water. Furthermore, excess irrigation can was nutrients out of the substrate and thus reduce nutrient availability. Thus, there is an intrinsic link between irrigation and fertilizer management in greenhouses.

The purpose of this paper is not to provide to provide an exhaustive review of greenhouse irrigation and fertilization research, but rather to provide an overview of the research being conducted at the University of Georgia into automation of irrigation and fertigation in greenhouses. Past and ongoing research is described here using summaries from three studies with different objectives. Study 1 looked at the

effect of θ on the growth and water use of petunia (*Petunia* x *hybrida*) and how that water use is affected by plant age and environmental conditions. The second study explores the physiological responses of plants to different θ levels, in an attempt to explain how θ affects growth of annual vinca (*Catharanthus roseus*). Finally, the third study discusses recent efforts in controlling both θ and EC using *in situ* sensors.

Study 1: Automated control of substrate water content and its effect on growth and water use of petunia

Soil moisture sensor controlled irrigation allows for precise control of θ (Nemali and van Iersel, 2006), and thus allows for the quantification of the effects of θ on plant growth. At the same time, by monitoring how much water us required to maintain a stable θ, it is possible to determine the evapotranspirational water loss from the plants. Such information can be used to precisely quantify crop water requirements, information that has been lacking for ornamental greenhouse crops. Therefore, the objectives of this study were to 1) determine the effects of different θs on the growth and water relations of petunia (*Petunia* × *hybrida*) and 2) to quantify the daily water use of petunias as they develop from seedlings to salable plants.

Materials and Methods

Plant material and growing conditions. Containers were filled with 10 L of a peat (60%) - perlite (40%) substrate (Fafard 2P, Fafard, Agawam, MA). Twenty four petunia 'Velvet Carpet' seedlings grown in 512-cell plug flats were transplanted into each container and watered in. The substrate was well-watered during the first nine days after transplanting to allow the seedlings to establish. At each irrigation throughout this study plants were watered with a water-soluble fertilizer solution at a N concentration of 200 $mg \cdot L^{-1}$ using a fertilizer injector .

Treatments. After plants were established, irrigation was controlled by an automated irrigation controller similar to the one described by Nemali and van Iersel (2006). Two capacitance sensors (EC-5, Decagon, Pullman, WA, USA) were inserted into the substrate in each container. The sensors were measured every 20 minutes using a datalogger (CR10, Campbell Scientific). Voltage readings from the soil moisture sensors were converted to θ ($m^3 \cdot m^{-3}$) using our own substrate-specific calibration (θ = voltage × 1.7647 - 0.4745, r^2 = 0.95). The readings from the two sensors in each container were then averaged, and the datalogger subsequently compared the average θ in each container with the θ threshold for that particular container. As soon as the average θ in a container dropped below the threshold for irrigation, the datalogger opened an irrigation valve for 1 min, applying 63 mL of fertilizer solution to the container. Irrigation thresholds were θ-values of 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, and 0.40 $m^3 \cdot m^{-3}$.

Data collection. In addition to measuring θ and controlling irrigation, the datalogger stored the θ readings from each sensor every two hours and counted the number of times each container was irrigated. This allowed us to calculate the number of irrigations each container received each day, and thus the daily irrigation volume. At the end of the experiment, the shoots were cut off at the substrate surface and dried for 1 week at 80 °C, after which their dry weight was determined. Environmental conditions including photosynthetic photon flux were collected throughout the study and *PPF* was used to calculate the daily light integral (DLI, total amount of *PPF* received during a day).

Experimental design and data analysis. The data were analyzed using regression analysis. To quantify the effects of plant age and DLI on daily irrigation volume, multiple regression was used to fit the following equation: daily water use = a_0 + a_1×DLI + a_2×time, where a_0, a_1, and a_2 are regression coefficients, and time is the

number of days since the start of the treatments. Only the data from the treatment with a θ threshold of 0.40 $m^3 \cdot m^{-3}$ were used because this treatment had the highest water use and the regression equation therefore describes the maximum amount of water that is used by these plants.

Results and Discussion

The automated irrigation system generally was able to maintain θ close to the θ threshold (Fig. 1). It took 9 d from the start of the treatments for the substrate to dry out to the lowest θ threshold (0.05 $m^3 \cdot m^{-3}$). From day 13 to 15, θ in one of the two plots with θ thresholds of 0.30 $m^3 \cdot m^{-3}$ greatly exceeded the threshold. This was caused by heavy rain on day 13, which caused water to leak through the greenhouse glazing and drip into that particular tray.

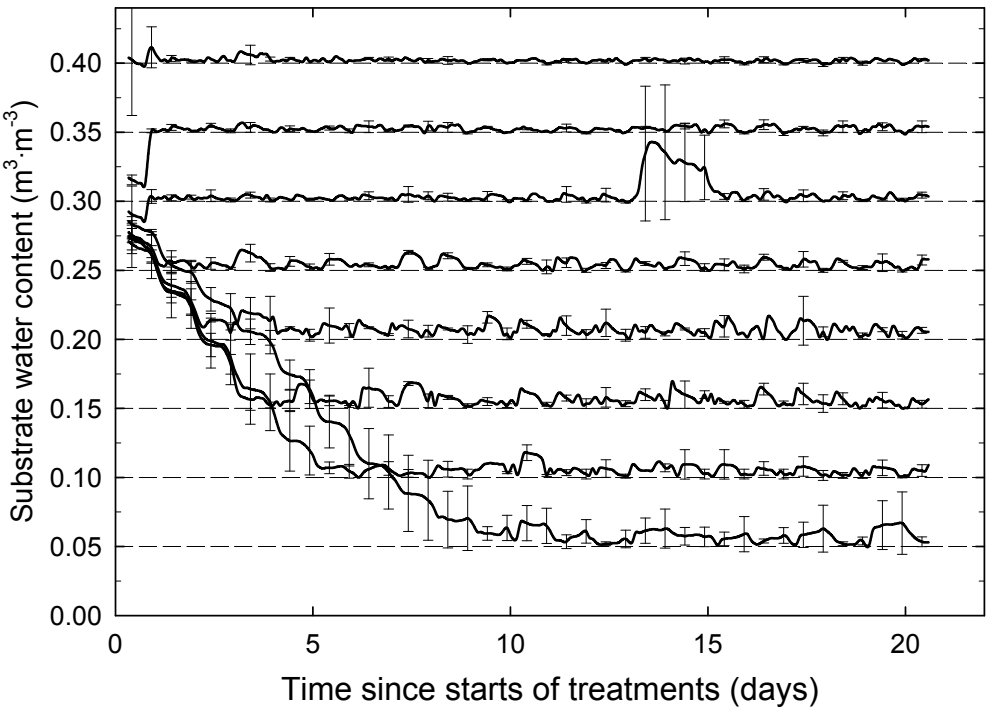


Fig. 1. Average substrate volumetric water content (θ) as maintained by a soil moisture sensor-controlled automated irrigation system. Dashed lines indicate the θ threshold at which the containers in the various treatments were irrigated.

The total amount of water applied during the treatment period increased linearly with increasing θ, from approximately 80 mL/plant in the 0.05 $m^3 \cdot m^{-3}$ treatment to 610 mL/plant in the 0.40 $m^3 \cdot m^{-3}$ treatment (Fig. 2). None of the applied irrigation water leached from the containers in this experiment.

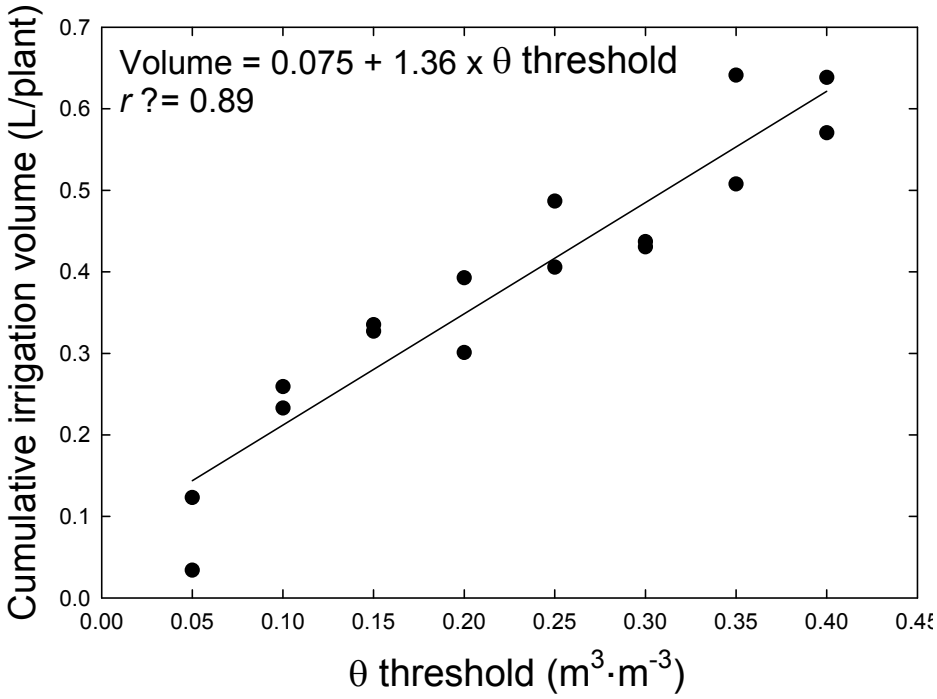


Fig. 2. Cumulative irrigation volume during the 22 d experiment, as a function of the substrate volumetric

The daily irrigation volume in the 0.40 $m^3 \cdot m^{-3}$ treatment was 12 – 20 mL/plant during the first week, and increased to a maximum of 44 mL/plant near the end of the experiment. As was the case with the total amount of water applied during the entire treatment period (Fig. 2), the daily irrigation volume increased with increasing θ thresholds (Fig. 3, bottom). In addition, irrigation volume in all treatments tended to increase during the experiment, likely because older, larger plants use more water. However, there were large daily fluctuations in the amount of irrigation water applied,

which were correlated with changes in DLI. For example, irrigation volumes were low on day 7 and 14, when DLI was low (Fig. 3). Using the data from the 0.40 $m^3 \cdot m^{-3}$ treatment for multiple regression confirmed that both time and DLI were positively correlated with daily irrigation volume. The regression equation [daily irrigation volume (mL/plant) = -7.15 + 1.14 × DLI + 1.54 × time, $r^2 = 0.79$] indicates that water use of the plants increased by 1.54 mL/d over the course of the study and by 1.14 mL/d for each additional $mol \cdot m^{-2}$ of *PPF*. This suggests that it may be possible to use

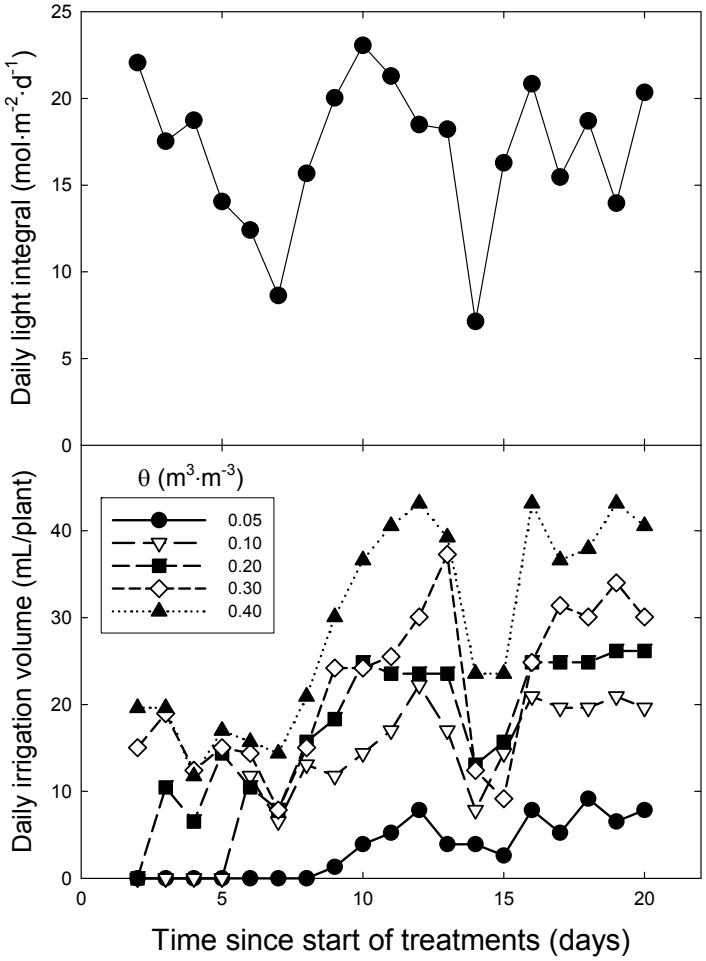


Fig. 3. Daily light integral (top) and daily irrigation volume (bottom) for petunias irrigated with a soil moisture sensor-controlled irrigation system. Substrate volumetric water content (θ) thresholds ranged from 0.05 to 0.40 $m^3 \cdot m^{-3}$. Daily irrigation volumes of only five of 8 treatments are shown for clarity.

relatively simple models to predict the water needs of floricultural crops. Similarly, daily water use of abutilon (*Abutilon* × *hybridum*) and lantana (*Lantana camara*) was strongly correlated with plant age and DLI (Kim and van Iersel, 2009). In Kim and van Iersel's study (2009), the daily maximum vapor pressure deficit also was correlated with daily water use, although not as strongly as either DLI or plant age. Overall, daily water use of abutilon and lantana could be accurately described as a function of time, DLI, and vapor pressure deficit (r^2 = 0.94; Kim and van Iersel, 2009). However, in both Kim and van Iersel (2009) and the current study, the data reflect the actual irrigation volume versus the predicted irrigation volume of the same plants (Fig. 4); *i.e.*, the predictions of daily water needs were not evaluated with an independent data set. For a more definitive evaluation of simple regression models to predict plant water needs, it will be necessary to grow plants for model development separate from those to be used for model evaluation to assure that the two data sets are independent (Wallach, 2006). Until such studies have been conducted, simple descriptive models like this should not be used to automate greenhouse irrigation. We are currently further pursuing this research.

There was a quadratic relationship between the θ threshold and shoot dry weight; as the threshold increased from 0.05 to 0.25 $m^3 \cdot m^{-3}$, shoot dry weight increased from approximately 0.7 g/plant to 1.7 g/plant. There was little additional increase in dry weight as the θ threshold further increased to 0.40 $m^3 \cdot m^{-3}$ (Fig. 4, left).

There was a linear relationship between the total irrigation volume and shoot dry weight (Fig. 4, right). The slope of the regression line is a measure of water use efficiency: for every liter of water applied an additional 2.54 g of shoot dry weight was produced; conversely, 394 mL of water was used for the production of 1 g shoot dry mass. Unlike most measures of water use efficiency, the slope of this regression line accounts for both evaporation from the substrate and transpiration from the plants.

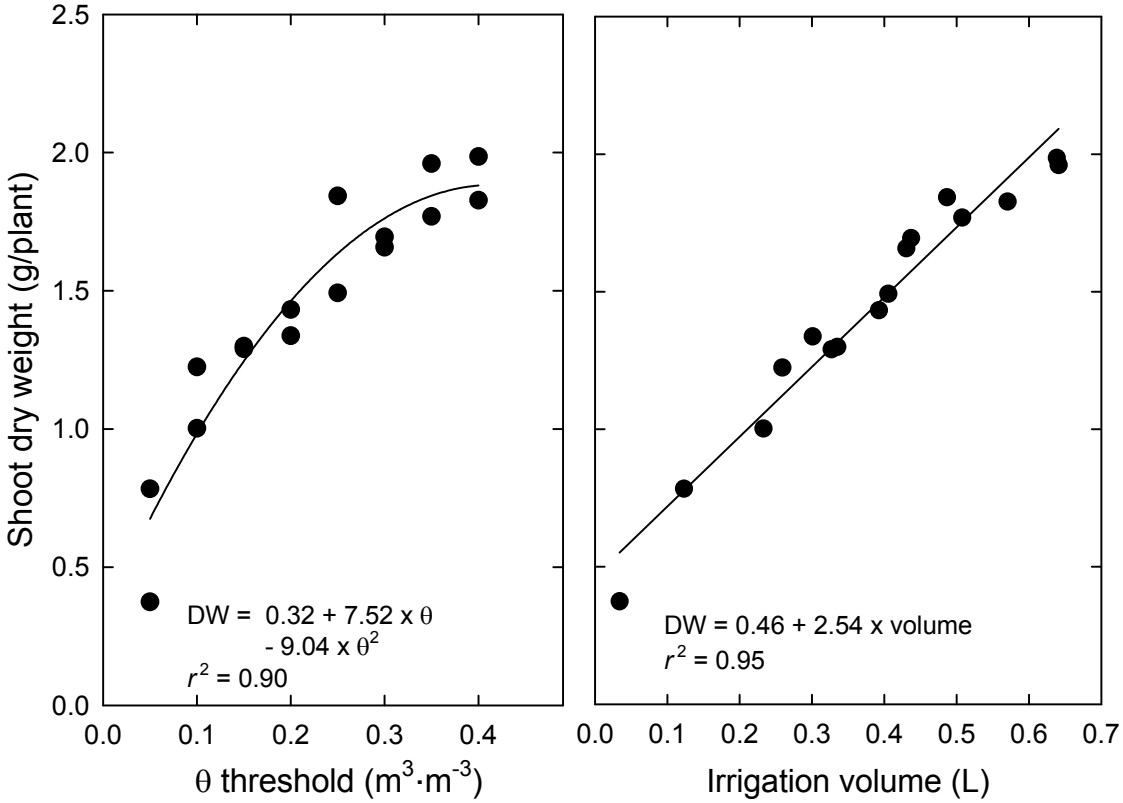


Fig. 4. Shoot dry weight (DW) of petunias irrigated with a soil moisture sensor-controlled automated irrigation system as a function of the substrate water content (θ) threshold (left) or total irrigation volume since the start of the irrigation treatments (right).

Study 2: Effects of substrate water content of growth and physiology of vinca (*Catharanthus roseus*)

The previously described study with petunia showed that differences in θ can have a pronounced effect on plant growth, but that study did not explore underlying physiological mechanisms that may cause these differences in growth. The objective of this research was to quantify growth and physiological responses of annual vinca (*Catharanthus roseus*) to different substrate water contents.

Materials and Methods

Plant material and treatments. Plug seedlings of vinca 'Titan Burgundy' were obtained from a commercial seedling producer and 24 seedlings were transplanted into containers (30 cm × 46 cm × 17 cm) filled with 10 L of a peat-perlite 60/40 v/v) growing medium (Fafard 2P; Fafard, Anderson, SC). At each irrigation plants were watered with a water-soluble fertilizer solution with a N concentration of 200 $mg \cdot L^{-1}$. To allow the seedlings to establish, the plants were kept well-watered (θ at least 0.47 $m^3 \cdot m^{-3}$) for one week, after which the plants were irrigated based on the θ. All plots were water using a soil moisture sensor-based, automated irrigation system, similar to that described for the petunia study. The set points for irrigation in this study were 0.05, 0.11, 0.17, 0.23, 0.29, 0.35, 0.41, or 0.47 $m^3 \cdot m^{-3}$.

Measurements. Leaf gas exchange parameters (transpiration rate, net photosynthesis, and stomatal conductance) were measured 35 d after the start of the treatments, and 7 d after the 0.05 $m^3 \cdot m^{-3}$ treatment had reached its set point, using a leaf photosynthesis system. Instantaneous water use efficiency was determined as the ratio between net photosynthesis and transpiration.

Leaf water and osmotic potential were measured using leaf cutter psychrometers at 22, 31, and 41 d after the start of the treatments. Turgor potential was measured as the difference between water and osmotic potential.

At the end of the study, 41 d after the start of the treatments, the area of the uppermost, fully expanded leaf of each plant was measured after which the shoots were cut off at the substrate surface and dried at 80 °C, after which shoot dry weight was determined.

Experimental design and statistical analysis. The experimental design was a randomized complete block with 8 treatments (θ set points) and two replications. Data were analyzed using regression analysis. An initial ANOVA indicated that there was no interaction between measurement day and treatment for either leaf water, osmotic, or turgor potential. Data were then averaged over the three measurements dates before further analysis.

Results and Discussion

As in the previously discussed petunia study, the irrigation system was able to maintain θ close to the set points. It took approximately three weeks for the substrate to dry to a water content of 0.05 m^3/m^3, but after the target substrate water content was reached, the water content remained stable throughout the experiment (Fig. 5).

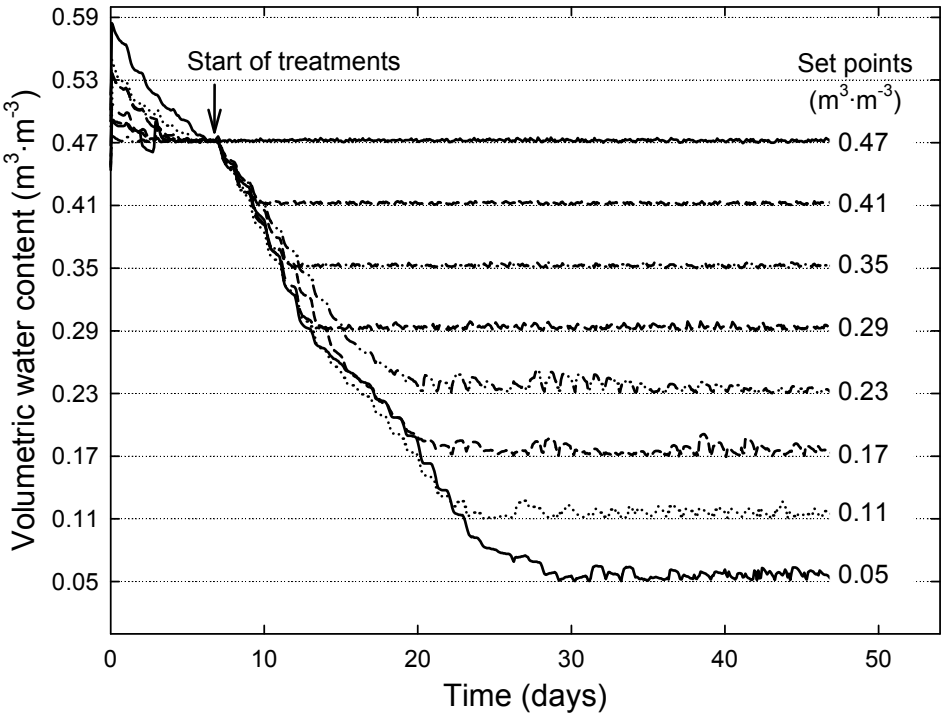


Fig. 5. Substrate water content throughout a seven week production period of annual vinca. Plants were watered only when the measured substrate water content dropped below the set point.

Plant growth was strongly correlated with θ. The shoot dry weight ranged from 0.6 g/plant at a θ of 0.05 m^3/m^3 to 1.8 g at a θ of 0.47 m^3/m^3 (Fig. 6, top).

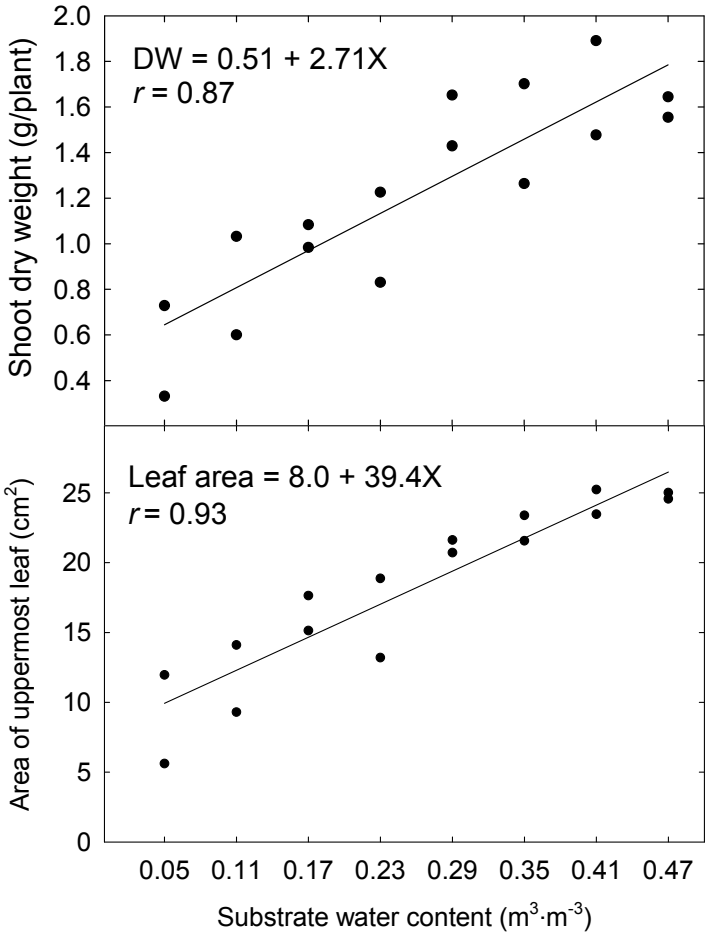


Fig. 6. The correlation between substrate water content and shoot dry weight (DW) (top) and the area of the uppermost fully expanded leaf (bottom) of vinca.

There also was a strong correlation between θ and the area of the uppermost fully expanded leaf, indicating that low θ inhibits leaf elongation. We did not find any evidence that this decrease in leaf elongation at low θ was due to low turgor in the leaves, since turgor potential was not affected by θ. This suggests that the decrease in leaf elongation instead may have been related to a change in cell wall properties. Both water and osmotic potential were decreased at low θ (Fig. 8).

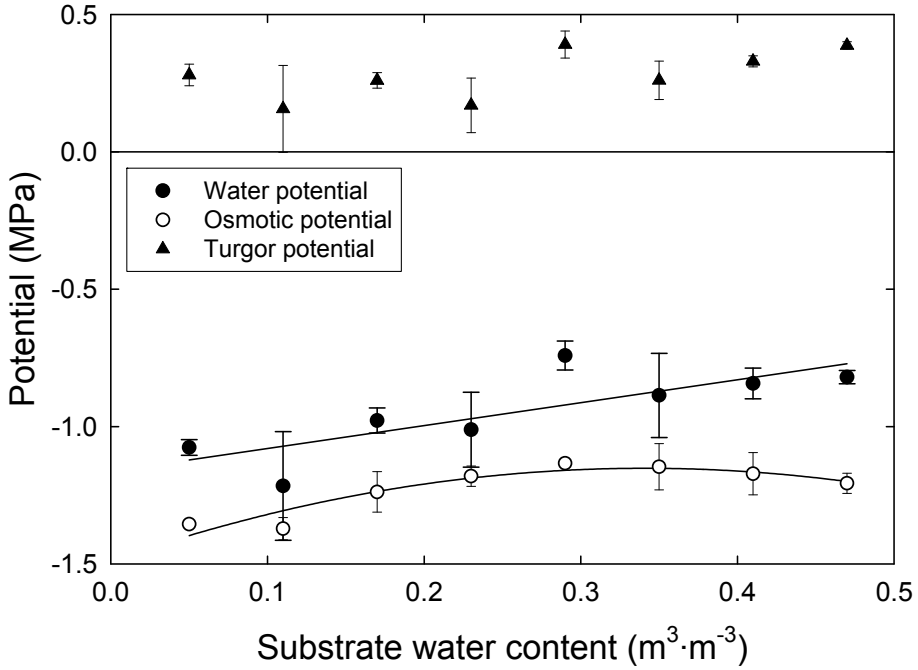


Fig. 7. The effect of substrate water content on the leaf water, osmotic, and turgor potential of vinca.

Leaf gas exchange data indicated decreased transpiration and stomatal conductance at low θ, but there was no significant effect on leaf photosynthesis (Fig. 8). The combined effects on transpiration and photosynthesis resulted in an increase in water use efficiency at low θ. Our finding that θ had no effect on leaf photosynthesis was a particularly interesting, since low levels of θ did reduce growth. Apparently this growth reduction was not due to decreased leaf photosynthesis. As a matter of fact, there was no correlation between leaf photosynthesis and plant dry weight (P = 0.49). Although this may seem surprising, it has long been known that there often is little or no correlation between leaf photosynthesis and plant growth (Evans, 1975). The main reasons for this are that leaf photosynthesis measurements do not take into account the total photosynthetically-active leaf area, the efficiency of conversion of photosynthates into dry matter, or the dry matter distribution within the plant (van Iersel and Bugbee, 2000).

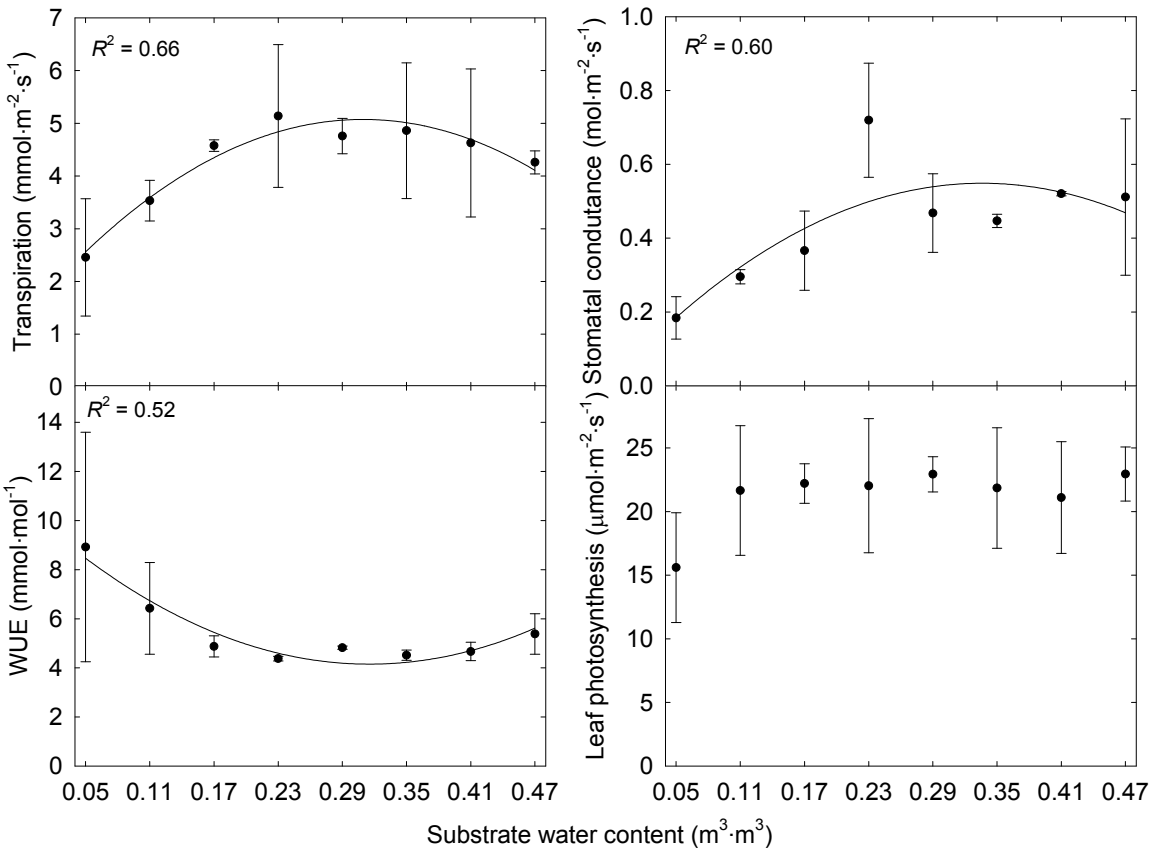


Fig. 8. The effect of substrate water content on transpiration, stomatal conductance, instantaneous water use efficiency, and photosynthesis of vinca leaves. Curves indicate statistically significant effects ($P < 0.05$). There was no significant effect on leaf photosynthesis ($P = 0.16$).

Unlike leaf photosynthesis, leaf area was strongly correlated with shoot dry weight ($r = 0.96$). These results suggest that decreased growth under drought stress was primarily the result of smaller leaves, and thus less photosynthetically active tissue, rather than decreases in the photosynthetic rate per unit leaf area. Likewise, we have seen that the growth of salt-stressed tomato plants also was better correlated with the area of the uppermost, fully-expanded leaf than with leaf photosynthesis (Montesano and van Iersel, 2007).

Overall, results from this study suggest that drought stress reduces growth, but that such growth reductions can occur without a significant reduction in growth. Instead the cause of the growth reductions appears to an inhibition of leaf elongation by drought stress, thus resulting in small leaves and less photosynthetically active tissue. The cause of the reduction in leaf elongation may a change in cell wall plasticity, rather than a decrease in turgor pressure.

Study 3: Automated Control of Water Content and Electrical Conductivity in Soilless Substrates: Proof of Concept

The first two studies clearly show that controlling irrigation with soil moisture sensors is feasible. However, irrigation and fertilization are close related processes in greenhouses, since the fertilizer generally is applied along with the irrigation water. The next step in this research therefore is to control both irrigation and fertilization by measure θ and electrical conductivity (EC) of the substrate. Electrical conductivity (EC) can be used as an indicator of the total amount of soluble salts in the substrate, and as such is commonly used as a proxy for nutrient availability. Recently, new sensors (5TE, Decagon Devices, Pullman, WA) have become available that can measure both the VWC and EC of soilless substrates. EC measurements are taken using two small stainless steel screws embedded in the sensor. These screws serve as the electrodes for the EC measurements. Thus, EC measurements using these sensors reflect the bulk EC of the substrate; i.e., these measurements reflect how well the substrate as a whole (including the solid substrate components, the pore water in the substrate, and the air in the substrate) conducts electricity. However from a plant's perspective, it is more relevant to determine the EC of the pore water. Hilhorst (2000) described a model that allows the estimation of pore water EC from measurements of bulk EC, temperature and the real portion of the dielectric permittivity of the substrate (ε_b) which is measured by the sensors. The objective of this study was to determine

whether these 5TE sensors can be used to control both the water content and the EC of soilless substrates.

Materials and Methods

An automated irrigation and fertigation system was built using 16 5TE sensors connected to a datalogger (CR10X, Campbell Scientific, Logan, UT), using a multiplexer (AM16/32, Campbell Scientific) (Fig. 1). Sensor readings of ε_b were converted to the volumetric water content (VWC) of the substrate, using our own calibration for the peat-perlite mix used in this study (VWC = $-0.0387\times\varepsilon_b^2 + 3.4436\times\varepsilon_b - 4.1848$, $R^2 = 0.99$; VWC is expressed here as % water content and was subsequently converted to units of m^3/m^3 by dividing by 100). Bulk EC, temperature, and ε_b measurements were used to calculate pore water EC, using Hilhorst's equation (Hilhorst, 2000), with an offset value (dielectric permittivity of the dry substrate) of 1.55.

The datalogger determined whether the measured VWC was below the VWC set point for a particular plot. If so, the datalogger then compared the calculated pore water EC to the EC set point. If the pore water EC was above the EC set point, the plants were irrigated with tap water, otherwise the plants were fertigated with a fertilizer solution with an EC of approximately 10 mS/cm. Such a high fertilizer EC was needed to see a detectable increase in pore water EC. The datalogger activated solenoid valves to either irrigate or fertigate each group of plants using two relay drivers (SDM16ACDC, Campbell Scientific). To achieve this, two separate irrigation systems were built, one that provided plants with tap water and another one that provided plants with fertilizer solution (Fig. 9). The plants were irrigated/fertigated using drip emitters connected to pressure compensated emitters (2 lph, Netafim, Tel Aviv, Israel). Each time the irrigation/fertigation for a particular plot was activated,

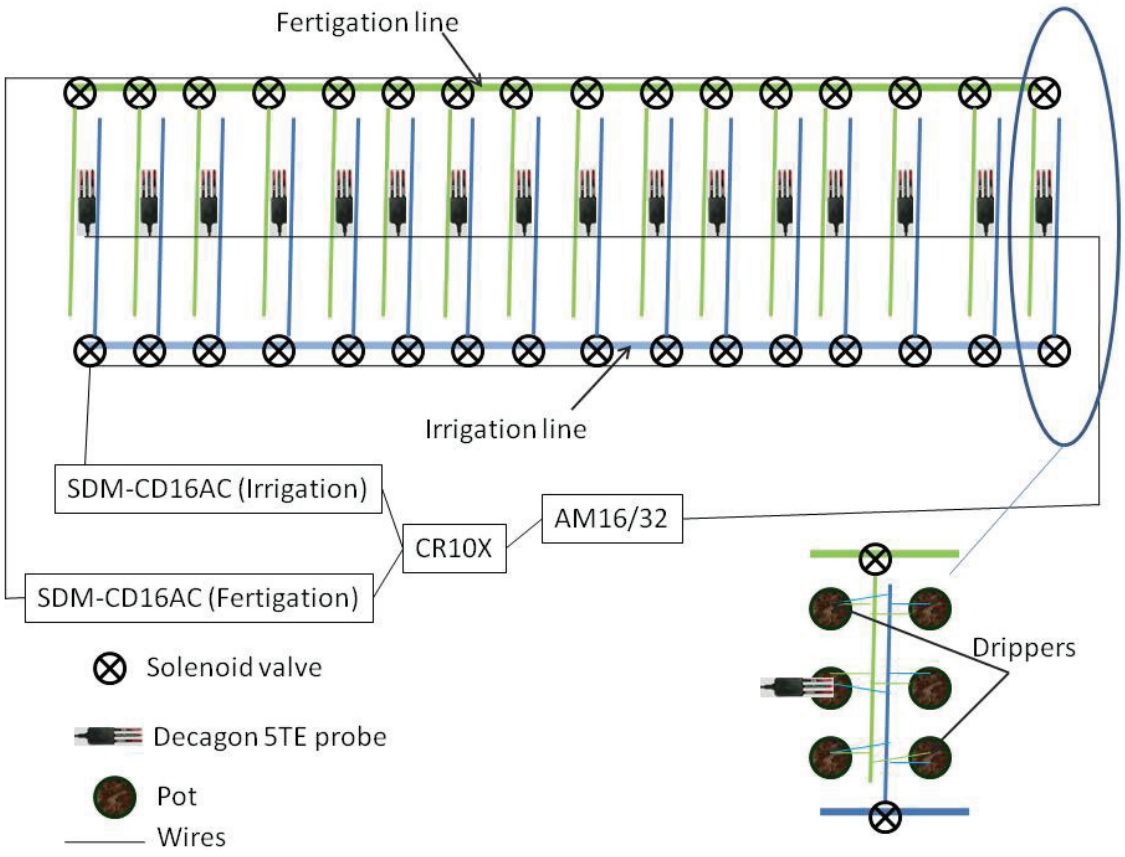


Fig. 9. Diagram of the sensor-controlled irrigation and fertigation system. The CR10X datalogger measures the water content and EC of the substrate using 5TE sensors and then determines whether to irrigate or fertigate a particular plot. Solenoid valves are activated using two relay drivers (SDM16ACDC). Only one out of six pots in each plot has a 5TE sensors. Irrigation and fertigation of all six plants in a plot are controlled based on the readings from that one sensor.

the solenoid valve opened for 30 s, thus applying approximately 16.5 ml per irrigation/fertigation event.

To test the automated irrigation and fertigation system, 96 ornamental cabbages (*Brassica oleracea)* were planted in 6" pots filled with a peat-perlite substrate (Fafard 2P, Agawam, MA, USA). The plants were arranged in 16 plots of 6 plants each, and a 5TE sensor was inserted into one of the six pots within each plot. The datalogger

was programmed to maintain the substrate water content at 0.25, 0.30, 0.35, or 0.40 m^3/m^3 and the pore water EC at 1, 2, 3, or 4 mS/cm in a factorial arrangement, thus resulting in 16 treatments. Due to the malfunctioning of one sensor, there are no data from the treatment with set points of 0.40 m^3/m^3 and 3 mS/cm. The shoots of the plants were harvested at the end of the study for dry weight measurements after 35 days.

Results and Discussion

Control of substrate VWC was good, with substrate VWC generally within 0.03 m^3/m^3 of the set point (Fig. 10). Control of pore water EC was more difficult, and there were large fluctuations in pore water EC, especially during the first 20 days of the experiment. Several factors contributed to the pore water EC fluctuations. 1) No control of pore water EC was possible until after the VWC of the substrates had reached the VWC set point, since plants were not irrigated or fertigated until this set point was reached. 2) The small screws that serve as EC sensors on the 5TE results in less than optimal contact between the electrodes and the substrate (or pore water). If there is an air pocket surrounding one of the electrodes, the measured bulk EC is low, resulting in a low calculated pore water EC as well. Good contact between the electrodes and the substrate is critical for the bulk EC measurements. Sensors with a different design, including larger electrodes, might therefore work better. 3) The calculated pore water EC depends on the VWC of the substrate, resulting in rapid changes in pore water EC following an irrigation or fertigation event. In addition, as the substrate dries out following irrigation, the pore water EC increases because the solution becomes more concentrated as the amount of water decreases. 4) There is no verification of how accurately the Hilhorst model determines the pore water EC, and recent research has suggested that the Hilhorst model may not be very accurate in soilless substrates (Arguedas Rodriguez, 2009). In addition, the correct offset value in

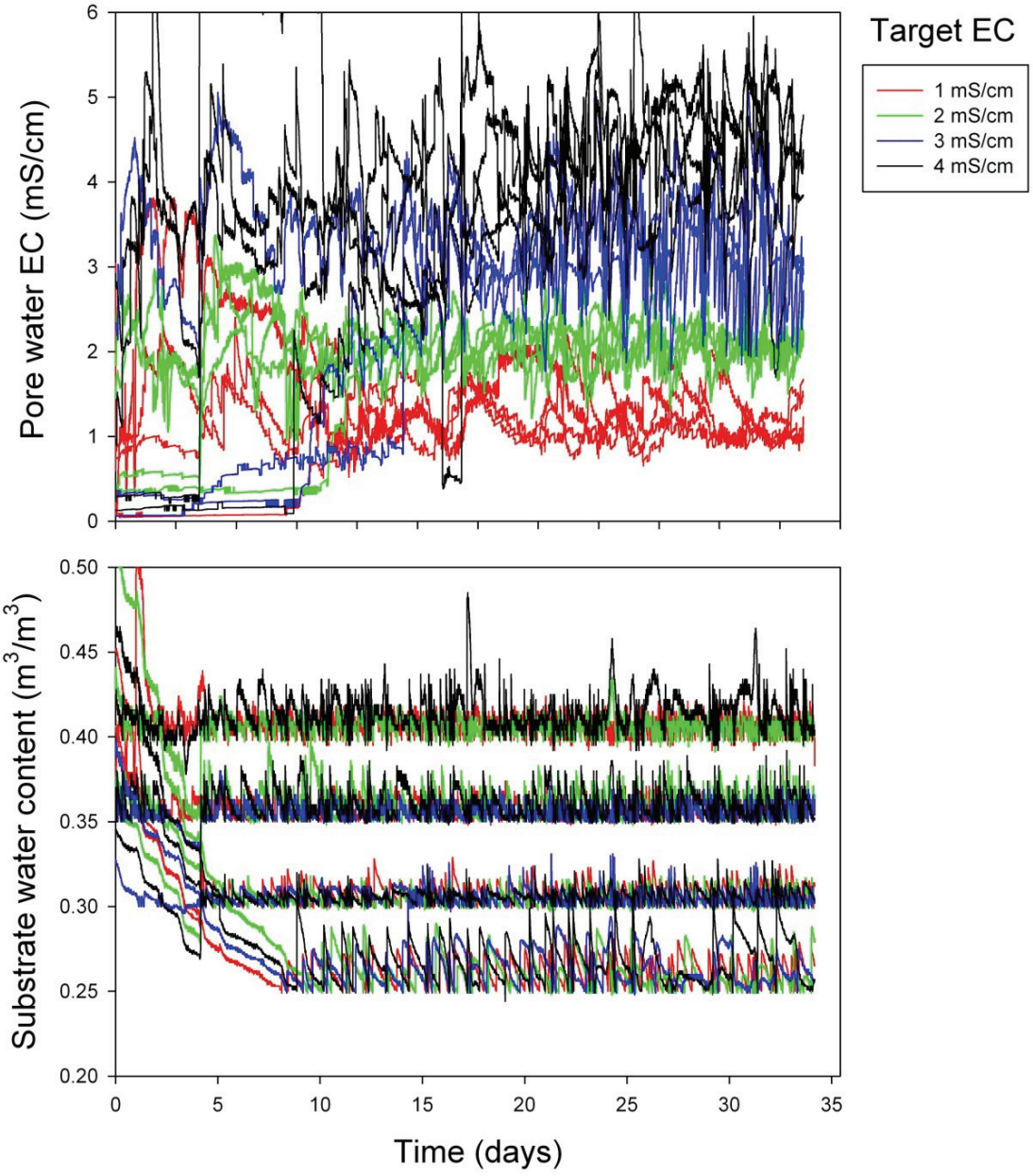


Fig. 10. Pore water EC (top) and water content (bottom) of the substrate throughout the experiment. Set points for pore water EC were 1, 2, 3, and 4 mS/cm, and those for substrate water content were 0.25, 0.30, 0.35, and 0.40 m^3/m^3. Note that control of substrate water content was better than control of pore water EC.

the Hilhorst model is not clear. More work is needed on how to determine pore water EC. 5) Fertilizer appears to move much more slowly through the substrate than water. Preliminary data showed that applying very small amounts of water (5.5 ml/plant) at a time resulted in increases in measured VWC, but not EC. Fertilizer concentrations were much higher at the location of the emitter applying the fertilizer solution than in the rest of the substrate. It may take several irrigation/fertigation events to move this fertilizer further into the substrate, where it can be measured by the EC sensor. To address this problem, we increased the amount of water applied at each irrigation, sacrificing some precision in control of VWC. Despite these issues, there were clear differences in the pore water EC among the different EC set points, and this was most evident during the last 15 days of the study. This suggests that the use of EC sensors for fertigation control is possible, but better sensors may be needed to get better measurements and control of pore water EC.

Depending on the treatment, the plants received between 0.14 and 3.6 liters of water and 0.26 and 1.15 liters of fertilizer solution during the 35-day experiment. For example, the plants in the treatment with set points of 1 mS/cm and 0.40 m^3/m^3 received 0.40 liters of fertilizer solution and 3.6 liters of water, while set points of 4 mS/cm and 0.30 m^3/m^3 resulted in applications of 1.15 liters of fertilizer solution and 0.35 liters of water during the experiment.

Both VWC and pore water EC set points affected the growth of the plants (Fig. 11). Shoot dry weight decreased as the set point for pore water EC increased. Shoot dry weight also was reduced by low set points for VWC, while shoot dry weights were similar with set points of 0.35 and 0.4 m^3/m^3. Such effects of VWC on plant growth are consistent with earlier findings (Burnett and van Iersel, 2008).

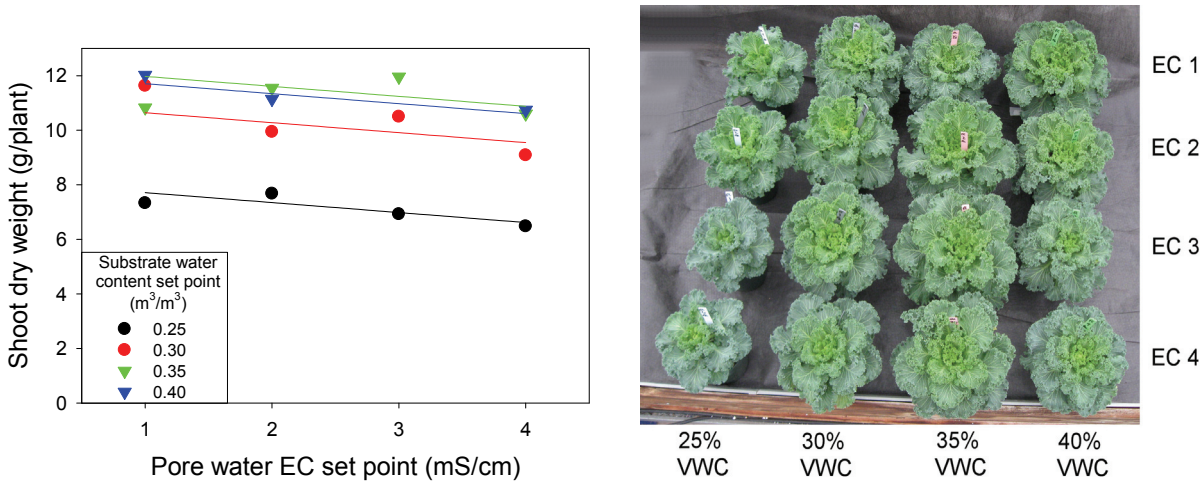


Fig. 11. The effect of set points for substrate volumetric water content (VWC) and pore water EC on the shoot dry weight of ornamental cabbage. There was a linear effect of pore water EC and a quadratic effect of substrate volumetric water content on shoot dry weight, but no interactive effect between pore water EC and VWC (Dry weight = - 30.5 - 0.366×EC + 234×VWC - 320×VWC^2, R^2 = 0.92, P < 0.0001). Representative plants from each treatment are shown on the right.

Acknowledgements

This research would not have been possible without the help of Krishna Nemali, Jongyun Kim, Francesco Montesano, Julian Miralles Crespo, Stephanie Burnett, and Sue Dove. Financial support for this research was provided by the Fred C. Gloeckner Foundation, the American Floral Endowment, the Center for Applied Nursery Research and a USDA-NIFA Specialty Crops Research Initiative Grant (Award no. 2009-51181-05768).

Literature cited

Arguedas Rodriguez, F. R. 2009. Calibrating capacitance sensors to estimate water content, matric potential, and electrical conductivity in soilless substrates. University of Maryland, MS Thesis. 118p.

Briggs, J., T. Whitwell, M. B. Riley, and T. Lee. 1998. Cyclic irrigation and grass waterways combine to reduce isoxaben losses from container plant nurseries. J. Environ. Hort. 16: 235-238.

Burnett, S. E. and M. W. van Iersel. 2008. Morphology and irrigation efficiency of *Gaura lindheimeri* grown with capacitance-sensor controlled irrigation. HortScience 43: 1555–1560.

Hilhorst, M. A. 2000. A pore water conductivity sensor. Soil Sci. Soc. Amer. J. 64: 1922-1925.

Howell, T. A. 2001. Enhancing water use efficiency in irrigated agriculture. Agron. J. 93: 281-289.

Jury, W. A. and H. Vaux. 2005. The role of science in solving the world's emerging water problems. Proc. Nat. Acad. Sci. 102: 15715-15720.

Kim, J. and M. W. van Iersel. 2009. Daily water use of abutilon and lantana at various substrate water contents. Proc. SNA Res. Conf. 54: 12-16.

Lea-Cox, J. and D. S. Ross. 2001. A review of the federal clean water act and the Maryland water quality improvement act: the rational for developing a water and nutrient management planning process for container nursery and greenhouse operations. J. Environ. Hort. 19: 226-229.

Jones, H. G. 2004. Irrigation scheduling: advantages and pitfalls of plant-based methods. J. Exp. Bot. 55: 2427-2436.

Jones, H. G. 2007. Monitoring plant and soil water status: established and novel methods revisited and their relevance to studies of drought tolerance. J. Exp. Bot. 58: 119-130.

Montesano, F., and M. W. van Iersel. 2007. Calcium can prevent toxic effects of Na^+ on tomato leaf photosynthesis, but does not restore growth. J. Amer. Soc. Hort. Sci. 132: 310-318.

Nemali, K. S. and M. W. van Iersel. 2006. An automated system for controlling drought stress and irrigation in potted plants. Scientia Hort. 110: 292–297.

Powell, C. C., and R. K. Lindquist. 1997. *Ball Pest and Disease Manual: Disease., Insect, and Mite Control on Flower and Foliage Crops*, 2nd edition. Ball Publishing, Batavia, Il.

Tyler H. H., S. L. Warren and T. E. Bilderback. 1996. Cyclic irrigation increases irrigation application efficiency and decreases ammonium losses. J. Environ. Hort. 14: 194–198.

van Iersel, M. W. and B. Bugbee. 2000. A semi-continuous, multi-chamber crop CO_2-exchange system: design, calibration, and data interpretation. J. Amer. Soc. Hort. Sci. 125: 86-92.

Energy-efficient production of greenhouse crops

Erik S. Runkle

Department of Horticulture, Michigan State University,

East Lansing, MI 48824, USA

Author for correspondence: runkleer@msu.edu

Abstract

Photoperiod, the photosynthetic daily light integral (DLI), and mean daily temperature are three environmental parameters that have the largest effects on plant growth and development. During commercial production of floriculture crops, one or more of these factors is often manipulated so that crops are marketable when desired. In temperate climates (e.g., >35 °N latitude), high-intensity (photosynthetic or supplemental) lighting is provided to increase growth and accelerate flowering when ambient light conditions are low. In addition, growers provide low-intensity (photoperiodic) lighting to deliver long days, which accelerates flowering of long-day plants. Finally, growers often control temperature, which influences cropping time and plant quality attributes. Since a substantial amount of energy is used to heat greenhouses located in cold climates, growers need to optimize temperature so that energy costs are minimized on a per-crop basis. This paper describes how light and temperature influence growth and flowering of floriculture crops and presents information to improve the energy efficiency of greenhouse crop production based heavily on recent research performed at Michigan State University.

Introduction

Floriculture crops are commonly grown in controlled greenhouse environments so that they are of desired quality and are ready for market on predetermined dates. Light and temperature primarily control growth and development and thus, are the two factors commonly manipulated during crop production. Light regulates photosynthesis, which controls plant growth attributes (shoot and root mass, flower bud number, etc.) but it can also influence extension growth and flowering. Temperature primarily controls plant rate of development and thus, has a strong effect on the duration that crops are in the greenhouse. A common goal of floriculture crop producers is to minimize the amount of time that crops are in the greenhouse, as well as to consume the least amount of energy for heating, especially when outdoor temperatures are low. Temperature is manipulated so that both of these considerations are minimized.

There are three dimensions of light that each influence plant growth and development. Light quantity or intensity refers to the instantaneous or cumulative amount of photosynthetic light received in a period of time (usually s or d) in a defined area (usually m^2) and is expressed in μmol or mol of photons (Thimijan and Heins, 1983). Light quantity primarily influences photosynthesis and thus root and shoot growth of plants. Light quality refers to the spectral distribution of light, for example, the ratio of red (R, 600 to 700 nm) to far red (FR, 700 to 800 nm) photons (Smith, 1994). Light quality can influence flowering and plant morphogenesis, such a stem extension and branching. Third, light duration refers to the photoperiod, or the duration of light and darkness each day. Many ornamental crops are sensitive to photoperiod with respect to flowering and thus, photoperiod is commonly truncated or increased to inhibit or promote flowering, whichever is desired.

In commercial greenhouse production of ornamental crops, supplemental lighting is sometimes provided to increase the photosynthetic daily light integral (DLI), depending primarily on growing location, time of year, and crop grown. Generally, the relative value of supplemental lighting is greatest for high-light (shade-avoiding) plants, in temperate climates from late autumn to early spring (e.g., when the DLI is <10 $mol \cdot m^{-2} \cdot d^{-1}$), at night or under cloudy conditions, and when plant density is high. Low-intensity lighting is even more widely used to shorten the dark period to promote flowering of long-day plants (LDP) or inhibit flowering of short-day plants (SDP).

Many factors are motivating growers to consider energy-efficient lighting strategies. For example, the incandescent bulb, which has been a common lamp type for photoperiodic lighting, is or will be unavailable in some countries because of its inefficiency at converting electricity into light. The cost of electricity in many countries also continues to increase, which makes operating lights more expensive. In addition, growers are increasingly conscious of their environmental footprint. Here, we highlight some of the recent research performed at Michigan State University that is focused on energy-efficient, effective, and economical production of floriculture crops.

Supplemental lighting

Commercial production of ornamentals typically consists of two distinct phases: the young plant (plug or liner) stage and the finish stage. When the ambient DLI is low, increasing the DLI with supplemental lighting is often cost-effective when producing young plants; lighting cost on a per-plant basis is low yet effects on growth and subsequent development can hasten crop timing. For example, mean shoot dry weight per internode increased as DLI increased from 4 to 12 $mol \cdot m^{-2} \cdot d^{-1}$ in five annual bedding plant species (Pramuk and Runkle, 2005b). In addition, subsequent

time to flower of all five species decreased as the DLI under which seedlings were grown increased. In a separate study, rooting and quality of New Guinea impatiens (*Impatiens hawkeri*) and petunia (*Petunia×hybrida*) cuttings increased and subsequent time to flower decreased as mean propagation DLI increased from ≈2 to 8-10 mol·m^{-2}·d^{-1} (Lopez and Runkle, 2008). During the finish plant stage, lighting costs often increase dramatically on a per-plant basis because plant density decreases.

Supplemental lighting during the entire production period of ornamentals is often economically infeasible because significant investments in lighting equipment and operation are required. For example, a simple estimated purchase and installation cost to provide supplemental lighting from 400-W high-pressure sodium (HPS) lamps was US$ 0.80 per μmol·m^{-2}·s^{-1} for each m^2 of greenhouse (Fisher and Both, 2004). In addition, an increase in rate of development of many annual ornamentals begins to saturate at a mean DLI of 12 to 15 mol·m^{-2}·d^{-1} (Blanchard, 2009). Thus, the value of providing supplemental lighting is diminished for many ornamental crops when the mean ambient DLI inside a greenhouse exceeds ≈15 mol·m^{-2}·d^{-1}. Therefore, growers with limited greenhouse lighting have queried at which young plant stage is supplemental lighting of most benefit.

In a recent study with petunia and pansy (*Viola ×wittrockiana*), seedlings were grown under a 16-h photoperiod with lighting from HPS lamps at 90 μmol·m^{-2}·s^{-1} (supplemental lighting) or 3 μmol·m^{-2}·s^{-1} (photoperiodic lighting; Oh et al., 2010). Seedlings remained under the lighting treatments for the entire production period (27 d for petunia and 33 d for pansy) or were exposed to the higher intensity for one-third or two-thirds of the seedling stage. In both species, supplemental lighting during the last two-thirds or the entire plug stage increased shoot dry weight and the number of leaves compared with supplemental lighting during the earlier stage or at the low intensity. In addition, supplemental lighting during the last two-thirds or the entire

plug stage accelerated flowering. Supplemental lighting provided for only one-third of the production period produced the greatest increase in shoot mass and leaf number when delivered during the last third period compared with the first or second period.

Photoperiodic lighting

There is a quantitative relationship between the irradiance (intensity and duration) of photoperiodic lighting and the flowering response of many LDP (Thomas and Vince-Prue, 1997). For example, flowering of lance coreopsis (*Coreopsis grandiflora*) and perennial rudbeckia (*Rudbeckia fulgida*) was increasingly promoted as the duration of night interruption (NI) lighting from incandescent lamps increased from 0.5 to 4 h during a 15-h skotoperiod (Runkle et al., 1998). A target minimum irradiance for photoperiodic lighting at plant height is 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$, but many species respond to a lower irradiance (Whitman et al., 1998). Flowering of some LDP is more rapid when low-intensity lighting contains FR light. The spectral distribution of light generally has less effect when photoperiodic lighting is provided to inhibit flowering of SDP.

Lighting from incandescent lamps, either at the end of the day or during the middle of the night, has been the traditional way that growers have shortened the night length. Incandescent lamps have been used extensively primarily because of their low purchase and installation costs. Strings of lamps can be hung above crops readily and can be moved with ease. The incandescent lamp emits light with a R:FR of ≈0.7, which is effective at inducing flowering in LDP and inhibiting flowering of SDP. There are also undesirable characteristics of incandescent lamps when used in the commercial production of ornamentals: they are only ≈10 to 12% efficient at converting electricity into photons within the 400 to 800 nm waveband and thus, they require a large electrical capacity (Thimijan and Heins, 1983). In addition, their low

R:FR promotes stem extension, which is often undesirable in the production of containerized plants.

To reduce energy consumption, incandescent lamps can be pulsed during the night (cyclic lighting) instead of operating continuously. For example, campanula (*Campanula carpatica*) and threadleaf coreopsis (*Coreopsis verticillata*) flowered comparably when the middle of a 15-h night was interrupted with a continuous 4 h with incandescent lamps, or when lamps were illuminated 20% of that time (6 min of light every 30 min for 4 h) (Runkle et al., 1998). In contrast, flowering of petunia and annual rudbeckia (*Rudbeckia hirta*) was delayed by 9 to 16 d when plants were grown at 20 ℃ under a 4-h, 20% cyclic NI lighting regimen compared with a continuous 4 h (Blanchard and Runkle, 2010).

Metal halide, HPS, and fluorescent lamps can also be effective at shortening the night length, particularly when used to extend the day (Whitman et al., 1998). These lamp types are more efficient at converting electricity into light, with ≥22% of the energy used to emit photons within the 400 to 800 nm waveband (Thimijan and Heins, 1983). In contrast with the incandescent lamps, the bulb lifespan of these three lamp types is negatively influenced by the on/off cycles and thus, they are usually not operated in a traditional cyclic manner. Given the high intensity of light from metal halide and HPS lamps, they can be widely spaced so that the minimum light intensity at crop height is ≥1 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Recently, we performed experiments to assess the efficacy of compact fluorescent (CF) lamps compared with incandescent lamps for providing long days to a variety of flowering annuals and herbaceous perennials (unpublished data). Plants were grown at 20 ℃ under a truncated 9-h photoperiod with or without a 6-h day extension to create a 15-h photoperiod, or a 2- or 4-h NI. Lighting was provided by incandescent (R:FR = 0.6) and CF lamps (R:FR = 8.4), alone or combined (R:FR =

0.9), at a photosynthetic photon flux (PPF) of 1.4 to 3.5 μmol·m^{-2}·s^{-1}. Time to flower of petunia was most rapid under incandescent lamps alone or alternating with CF lamps. In contrast, incandescent and CF lamps were similarly effective at inducing flowering of annual rudbeckia and inhibiting flowering of chrysanthemum (*Chrysanthemum*×*grandiflorum*). We conclude that, given their relative energy efficiency, CF lamps are, in some respects, an appealing replacement to incandescent lamps for many, but not all, photoperiodic crops.

An alternative photoperiodic lighting strategy is to use HPS lamps that either move over crops (e.g., on irrigation booms) or have a rotating reflector that casts a beam of light across crops (rotating HPS lighting). Although greenhouse growers have reported successful use of "boom lighting", to our knowledge, no scientific studies have provided a foundation for lighting guidelines or recommendations, such as how lighting frequency, intensity, and duration of irradiance interact to achieve desirable results. Growers have reported successful use of boom lighting with HPS or mercury lamps mounted approximately 3 to 4 m apart and 1 to 2 m above the crop. Generally, the lamps and booms are operated for 4 to 6 h during the middle of the night with a goal of illuminating plants at least once every 30 min.

We recently evaluated the effectiveness of rotating HPS lighting to inhibit flowering of SDP and promote flowering of LDP. The experiment was performed during natural short days and one 600-W rotating HPS lamp was mounted at one end of the greenhouse and operated for 4 h as NI lighting. Plants were grown at lateral distances of 1 to 13 m from under the rotating HPS lamp, which provided a maximum PPF of 25.4 to 0.3 μmol·m^{-2}·s^{-1}, respectively. Compared with a constant 4-h NI with incandescent lamps, flowering of lance coreopsis and annual rudbeckia was delayed at all lateral distances from under the HPS lamp (Blanchard and Runkle, 2010). In campanula and petunia, plants within 7 m and 10 m, respectively, from under the

rotating HPS lamp flowered at a similar time as plants under the 4-h incandescent NI treatment. Petunia within 10 m from under the rotating HPS lamp flowered earlier than under the 20% cyclic lighting, whereas flowering time of campanula was similar under the cyclic lighting as plants up to 10 m from under the rotating HPS lamp. In florists' (potted) chrysanthemum, the rotating HPS lamp was only effective at preventing flowering when plants were within 7 m from under the lamp (Blanchard and Runkle, 2009).

Generally, commercial growers have reported successful use of rotating HPS lamps to regulate flowering of photoperiodic ornamentals. In agreement with our research results, growers have reported that flowering is uneven and delayed in some long-day crops when the rotating HPS lamps are positioned too far apart. In addition, in our experiment with one lamp, chrysanthemum plants that were >7 m from the lamp had vegetative shoots on the side facing the lamp and reproductive shoots on the side opposing the lamp. Because we only used one lamp in this study, it is difficult to make spacing guidelines for rotating HPS lamps because most commercial growers would install multiple lamps and thus, plants could receive light from two or more fixtures.

From a commercial greenhouse grower's perspective, goals of photoperiodic lighting are that (1) it is sufficiently effective to control plant development, (2) purchase and installation costs are low, and (3) electricity costs for operation are low. To help address this later goal, we estimated weekly electricity consumption for six photoperiodic lighting strategies for 140 m^2 of greenhouse space, which is an estimate for the space that one rotating 600-W HPS lamp can sufficiently cover. However, as discussed previously, some of these lighting strategies were more effective than others on some crops. Comparisons were made with light from incandescent, CF lamps, and HPS lamps. The primary drawback of continuous 4-h NI lighting from incandescent

lamps is clear. The rotating HPS lamp is estimated to consume only 17% of the electrical consumption as the continuous 4-h NI with incandescent lamps, while electrical consumption for 25% cyclic incandescent lighting was comparable to constant 4-h lighting from CF or HPS lamps.

Future lighting considerations

At least in North America, growers in temperate climates continue to invest in lighting systems and learn how to cost-effectively manage their use. In the production of ornamentals, investing in photoperiodic lighting is typically much less expensive because of the number of floriculture species that are photoperiodic, the comparatively low purchase and installation costs, and the potential to reduce crop production time by a week or more. Ornamental growers in North America use supplemental lighting much less frequently, primarily because of the high investment costs and the challenge of obtaining a higher price for a higher quality plant. However, many of the more sophisticated growers in northern latitudes have installed HPS lamps, particularly in the production of rooted cuttings and seedlings, for reasons discussed previously.

Growers and scientists alike have had increasing interest in the development and potential use of light-emitting diodes (LEDs) for greenhouse applications, especially since 2008. Review articles about the virtues, opportunities, and limitations of LEDs in horticulture have been published (Massa et al., 2008; Morrow, 2008). An increasing number of companies produce and/or market LEDs for plant applications. Most companies are focusing on developing high-intensity lighting from LEDs to increase plant photosynthesis. The following criteria should be considered by lighting manufacturers and greenhouse growers alike:

- High electrical efficiency. This is a major reason why LEDs are so promising. Although current LEDs are only as electrically efficient as HPS lamps, their efficiencies are improving rapidly and will soon be more efficient than HPS lamps at converting electrical energy into photons of light. This efficiency can be quantified as micromoles of photosynthetic light ($\mu mol \cdot m^{-2} \cdot s^{-1}$) per watt of energy per area ($W \cdot m^{-2}$); the higher the value, the more efficient the lamp.

- High relative quantum efficiency (RQE). The RQE describes the efficiency of particular wavelengths of photons at driving photosynthesis (McCree, 1971; Fig. 1). Red light had the highest RQE, meaning that it was the most effective at eliciting photosynthesis. Blue light is about 70 to 75% as effective as red light, and green light is even less effective.

- Uniform distribution of light. Greenhouse lighting must be evenly distributed to ensure uniform crop response.

- Durable and reliable. LED arrays must be engineered and constructed to optimize light output, be of high quality, and be able to withstand extreme greenhouse conditions. For example, heat must be dissipated from LEDs sufficiently so that they operate at a favorable temperature; otherwise, their useful life can decrease.

- Compact size. Arrays should be as compact as possible so that shading to crops is minimized.

- Acceptable cost. The cost of these new technologies must be low enough that their purchase price can be offset by electricity savings or improved crop growth within a reasonable period of time (e.g., within 5 years). Because many greenhouses use supplemental lighting for only 3 or 4 months of the year, returns on investment can be longer. However, mass production and sales will rapidly reduce costs of equipping greenhouses compared to initial prototype models.

- Acceptable effect of light quality on plant morphology. The spectral output of lamps can influence plant height and chlorophyll concentration. Therefore, effects of lighting on plant morphology and appearance must also be considered.

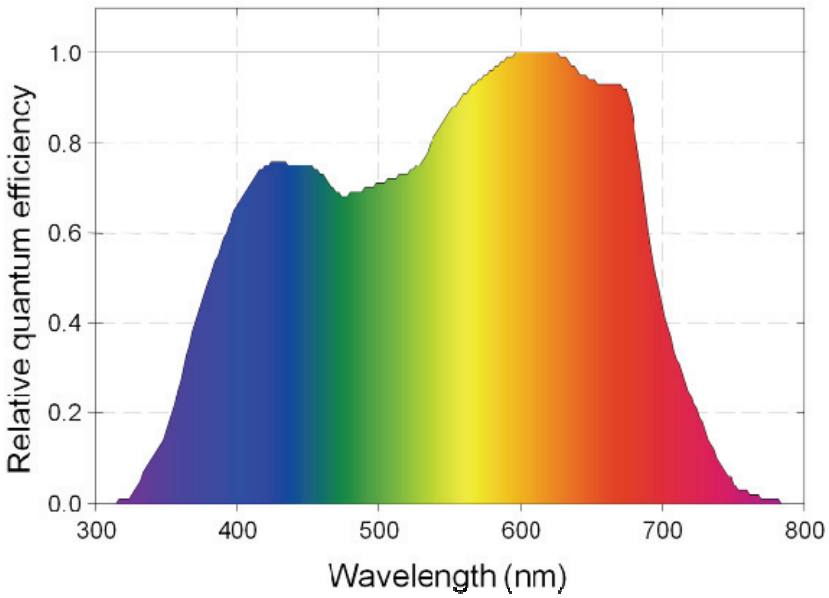


Fig. 1. The relative quantum efficiency curve developed by McCree (1971), which compares the efficacy of different wavelengths of light at eliciting photosynthesis.

Mean daily temperature

Temperature is the most commonly manipulated environmental factor in greenhouse production. The growing temperature used by commercial growers depends on numerous factors including: time of year, greenhouse location, environmental control systems, fuel cost for heating, crop finish dates, desired plant quality and size, stage of development, and plant species. Temperature also influences plant quality parameters such as flower number and size, branching, and mass. For example, flower bud number and flower size of lance coreposis, blanket

flower (*Gaillardia* ×*grandiflora*), and Shasta daisy (*Leucanthemum* ×*superbum*) increased as average daily temperature decreased from 27 °C to 15 °C (Yuan et al., 1998).

Efficient and predictable commercial production of greenhouse crops requires information on how species respond to the environment so that they can be accurately scheduled to finish for predetermined market dates. Studies have been performed on many economically important floriculture crops to quantify how light and temperature influence growth and development. Data from these experiments have been used to generate crop models that predict how changing environmental factors, such as mean daily temperature (MDT), affect the rate of plant development. Several analytical approaches have been described to model plant development rate in response to environmental conditions.

Plant developmental responses to temperature, such as flowering or leaf unfolding rate, are primarily controlled by the integrated mean daily temperature (MDT). The time required for the completion of a developmental event (e.g., time to unfold one leaf or time to anthesis) can be converted to a rate by calculating the reciprocal of time (e.g., 1/days). The parameters of the model are specific to a genotype or a development stage (Summerfield et al., 1991). In this model, the relationship between MDT and development rate is linear between the base temperature (T_{min}) and optimum temperature (T_{opt}). T_{min} is the temperature at or below which the rate of progress toward a developmental stage is zero and varies considerably among plant species (Roberts and Summerfield, 1987). T_{opt} is defined as the temperature at which the rate of progress toward a developmental event is maximal and also is species specific. Polynomial and exponential equations have also been used to describe the influence of MDT on development rate. The rate of plant development can also be secondarily influenced by other environmental factors, and

multiplicative models have been developed that combine factors such as MDT, photoperiod, and/or DLI. For example, we have developed such models for several ornamental annual crops (Moccaldi and Runkle, 2007; Pramuk and Runkle, 2005a), and models for >20 ornamental species are currently under development (Blanchard, 2009).

Temperature can also influence flower initiation and development. For example, temperature is the primary factor that controls inflorescence initiation and development of *Phalaenopsis* orchids. Undifferentiated buds at the base of each node remain dormant indefinitely when exposed to constant temperatures of ≥27 ºC (Sakanishi et al., 1980). After at least three weeks of exposure to temperatures <26 ºC, one or more of these buds can elongate and become an inflorescence (Sakanishi et al., 1980; Lee and Lin, 1984). For example, plants uniformly initiated inflorescences when grown at day/night temperatures of 25/20 ºC or 20/15 ºC for 4 to 5 weeks.

Constant and fluctuating day/night temperatures have similarly promotive effects on inflorescence initiation of *Phalaenopsis* when both are <26 ℃; a day/night temperature fluctuation does not accelerate flowering (Blanchard and Runkle, 2006). However, daily or periodic exposure to temperatures ≥26 ℃ can delay or completely inhibit flowering. High temperature flowering inhibition is apparently dependent on the cultivar, plant maturity, and the timing, duration, frequency, and magnitude of the high temperature. For example, inflorescences did not emerge from *Phalaenopsis* Miva Smartissimo × Canberra '450' or Brother Goldsmith '720' plants within 20 weeks when grown at a 12-h day temperature of 29 ℃ and a night temperature of 17, 23, or 29 ℃ (Blanchard and Runkle, 2006).

High-temperature inhibition of the emergence of an inflorescence was first reported by Sakanishi et al. (1980) on *Phalaenopsis amabilis*: 84% of plants exposed to 28 ℃ for 12 h developed an inflorescence, whereas only 46% of plants developed

an inflorescence when the high temperature was delivered for 14 h. Flowering of *Doritaenopsis* 'Lava Glow' plants was completely prevented when grown at a 12-h day temperature of 30 ℃ and night of 20 ℃ (Wang, 2007). Interestingly, none of the plants grown at a 12-h day/night of 25/30 ℃ or 30/25 °C flowered. In *Doritaenopsis* 'Newberry Parfait', 80% of plants flowered when exposed to 8 h at 29 ℃ each day (the remaining time was at 20 ℃), but time to flower was 7 to 8 weeks later than that of plants at a constant 20 ℃ (Newton and Runkle, 2009).

Once an inflorescence has initiated, time to the first open flower is dependant on the variety and mean temperature. However, if a plant with an inflorescence <10 cm is subsequently grown at ≥28 ℃, an inflorescence can elongate indefinitely without flowering, flower buds can abort, and a vegetative plant can develop on the inflorescence instead of flower buds (Sakanishi et al., 1980). At temperatures ≤26 ℃, time to appearance of an inflorescence and time to flower is increasingly delayed as mean temperature decreases. For example, time from inflorescence appearance to first open flower of two *Phalaenopsis* cultivars was delayed by 9 to 17 d as temperature decreased from 23 to 20 ℃ (Blanchard and Runkle, 2006). In addition, inflorescence and flower bud number were generally greater when *Phalaenopsis* were grown at lower temperatures (e.g., ≤20 ℃; Lee and Lin, 1984; Blanchard and Runkle, 2006).

Greenhouse temperature and energy consumption

In temperate climates, high energy inputs can be required to maintain a desirable greenhouse temperature, making fuel for heating one of the largest floriculture production expenses (Bartok, 2001). In the Netherlands, greenhouses account for 79% of the energy used by the agricultural sector and 7% of the country's total energy consumption (Lansink and Ondersteijn, 2006). Rising and volatile fuel costs for

heating greenhouses have motivated many growers to improve energy conservation and to minimize energy inputs for greenhouse crop production.

Models that predict the effect of temperature on plant development can be used to estimate crop production time periods at a range of greenhouse temperatures. For example, petunia 'Wave Purple' grown under a long day takes approximately 64 days from transplant of a seedling until first flowering at an MDT of 14 ℃, whereas time to flower is less than half that time at 23 ℃ (Fig. 2).

The Virtual Grower computer program can be used to estimate greenhouse heating costs in the United States. The program was developed by Jonathan Frantz and colleagues at the USDA-ARS in Toledo, Ohio and is available free at www.virtualgrower.net. Version 2.6 was released in June 2010 and the program continues to be developed. Once the program is downloaded and installed, the user can create a virtual greenhouse and enter information such as their location, greenhouse dimensions and design, greenhouse construction materials, heating system characteristics, fuel type and price, heating schedule, and presence and type of energy curtain. That information is used to estimate heating costs per square foot per month. By changing one or more variables, such as planting date or temperature setpoint, one can predict the effect on fuel costs. For example, using the scheduling information of petunia 'Wave Purple' from Fig. 2, the estimated heating cost to produce plants in flower on April 1 in Grand Rapids, Michigan at 17 ℃ is $0.45 per square foot of production space (Fig. 3). If transplanted later and grown at 23 ℃, the estimated heating cost is reduced by 11% to $0.40 per square foot. As expected, heating costs are lower for a greenhouse with the same characteristics but located in a warmer climate. Thus, with adequate crop information and software such as Virtual Grower, growers can identify a temperature regimen that consumes the least amount of energy on a per-crop basis.

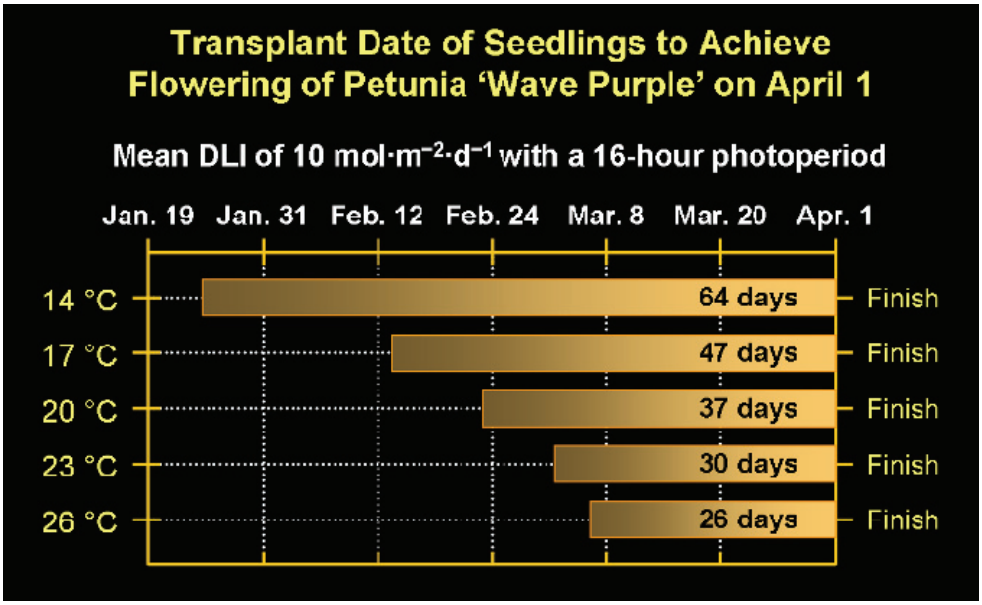


Fig. 2. The effect of mean daily temperature on crop timing and estimated planting date of petunia 'Wave Purple' so that plants are in flower on April 1.

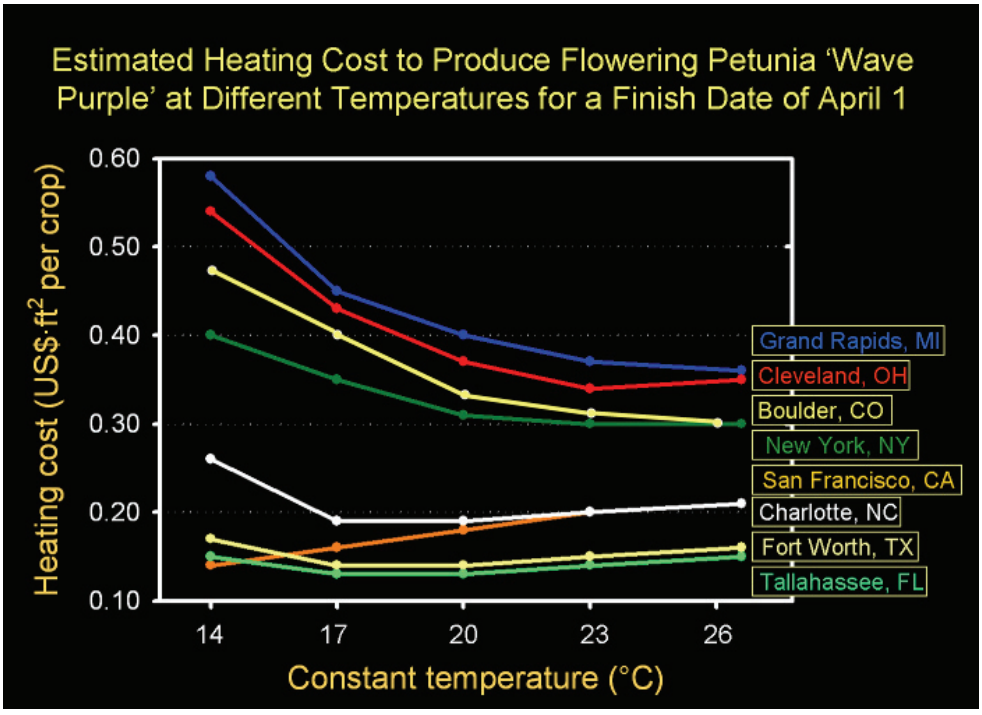


Fig. 3. Estimated heating costs to produce flowering petunia 'Wave Purple' for April 1 at a range of locations in the United States using Virtual Grower software.

Literature cited

Bartok, J. W. Jr. 2001. Energy conservation for commercial greenhouses. Natural Resource, Agriculture, and Engineering Service Cooperative Extension, Ithaca, NY.

Blanchard, M. G. 2009. Manipulating light and temperature for energy-efficient greenhouse production of ornamental crops. Ph.D. thesis. Michigan State Univ., East Lansing.

Blanchard, M. G. and E. S. Runkle. 2010. Intermittent light from a rotating high-pressure sodium lamp promotes flowering of long-day plants. HortScience 45: 236-241.

Blanchard, M. G. and E. S. Runkle. 2006. Temperature during the day, but not during the night, controls flowering of *Phalaenopsis* orchids. J. Expt. Bot. 57:4043-4049.

Blanchard, M. G. and E. S. Runkle. 2009. Use of a cyclic high-pressure sodium lamp to inhibit flowering of chrysanthemum and velvet sage. Scientia Hort. 122: 448-454.

Fisher, P. and A. J. Both. 2004b. Supplemental Lighting Technology and Costs. p. 43-46. In: P. Fisher and E. Runkle (eds.), Lighting Up Profits: Understanding Greenhouse Lighting. Meister Media Worldwide, Willoughby, Ohio.

Lansink, A. O. and C. Ondersteijn. 2006. Energy productivity growth in the Dutch greenhouse industry. Amer. J. Agr. Econ. 88: 124-132.

Lee, N. and G. M. Lin. 1984. Effect of temperature on growth and flowering of *Phalaenopsis* white hybrid. J. Chinese Soc. Hort. Sci. 30: 223-231.

Lopez, R. G. and E. S. Runkle. 2008. Photosynthetic daily light integral during propagation influences rooting and growth of cuttings and subsequent development of New Guinea impatiens and petunia. HortScience 43: 2052-2059.

Massa, G. D., H. H. Kim, R. M. Wheeler, and C. A. Mitchell. 2008. Plant productivity in response to LED lighting. HortScience 43: 1951-1956.

McCree, K. J. 1961. The action spectrum, absorptance, and quantum yield of photosynthesis in crop plants. Agric. Meteorol. 9: 191-216.

Moccaldi, L. A. and E. S. Runkle. 2007. Modeling the effects of temperature and photosynthetic daily light integral on growth and flowering of *Salvia splendens* and *Tagetes patula*. J. Amer. Soc. Hort. Sci. 132: 283-288.

Morrow, R. C. 2008. LED Lighting in Horticulture. HortScience 43: 1947-1950.

Newton, L. A. and E. S. Runkle. 2009. High-temperature inhibition of flowering of *Phalaenopsis* and *Doritaenopsis* orchids. HortScience 44: 1271-1276.

Oh, W., E. S. Runkle, and R. M. Warner. 2010. Timing and duration of supplemental lighting during the seedling stage influence quality and flowering in petunia and pansy. HortScience (in press).

Pramuk, L. A. and E. S. Runkle. 2005a. Modeling growth and development of celosia and impatiens in response to temperature and photosynthetic daily light integral. J. Amer. Soc. Hort. Sci. 130: 813−818.

Pramuk, L. A. and E. S. Runkle. 2005b. Photosynthetic daily light integral during the seedling stage influences subsequent growth and flowering of *Celosia*, *Impatiens*, *Salvia*, *Tagetes*, and *Viola*. HortScience 40: 1336-1339.

Roberts, E. H. and R. J. Summerfield. 1987. Measurement and prediction of flowering in annual crops, p. 17-50. In: Atherton, J.G. (ed.). Manipulation of flowering. Butterworths, Kent, UK.

Runkle, E. S., R. D. Heins, A. C. Cameron, and W. H. Carlson. 1998. Flowering of herbaceous perennials under various night interruption and cyclic lighting treatments. HortScience 33: 672-677.

Sakanishi, Y., H. Imanishi, and G. Ishida. 1980. Effect of temperature on growth and flowering of *Phalaenopsis amabilis*. Bull. Univ. Osaka Prefecture, Series B: Agri. Biol. 32: 1-9.

Smith, H. 1994. Sensing the Light Environment: The Functions of the Phytochrome Family. p. 377-416. In: R.E. Kendrick and G. H. M. Kronenberg (eds.), Photomorphogenesis in Plants 2nd ed. Dordrecht, The Netherlands, Kluwer Academic Publ.

Summerfield, R. J., E. H. Roberts, R. H. Ellis, and R. J. Lawn. 1991. Towards the reliable prediction of time to flowering in six annual crops. I. The development of simple models for fluctuating field environments. Expt. Agr. 27: 11-31.

Thimijan, R. W. and R. D. Heins. 1983. Photometric, radiometric, and quantum light units of measure: A review of procedures for interconversion. HortScience 18: 818-822.

Thomas, B. and D. Vince-Prue. 1997. Photoperiodism in Plants, 2nd ed. Academic Press, London.

Wang, Y. T. 2007. Average daily temperature and reversed day/night temperature regulate vegetative and reproductive responses of a *Doritis pulcherrima* Lindley Hybrid. HortScience 42: 68-70.

Whitman, C. M., R. D. Heins, A. C. Cameron, and W. H. Carlson. 1998. Lamp type and irradiance level for daylength extensions influence flowering of *Campanula carpatica* 'Blue Clips', *Coreopsis grandiflora* 'Early Sunrise', and *Coreopsis verticillata* 'Moonbeam'. J. Amer. Soc. Hort. Sci. 123: 802-807.

Yuan, M., W. H. Carlson, R. D. Heins, and A. C. Cameron. 1998. Effect of forcing temperature on time to flower of *Coreopsis grandiflora*, *Gaillardia* ×*grandiflora*, *Leucanthemum* ×*superbum*, and *Rudbeckia fulgida*. HortScience 33: 663-667.

Trends of *Phalaenopsis* orchid breeding research

Fure-Chyi Chen*, Shih-Wen Chin

Department of Plant Industry, National Pingtung University of Science & Technology, Taiwan

* Author for correspondence : furechen@mail.npust.edu.tw

Abstract

Phalaenopsis is one of the most popular and important orchids produced at commercial scale. New cultivars are produced by selection of seedling plants during their flowering stage. After selection of orchid plants with desirable traits, tissue culture and mass production of young plantlets were achieved through flower stalk node culture in vitro. Cross hybridization by artificial pollination among different parental hybrids usually can lead to capsule set and seed formation. However, breeding barriers are present in Phalaenopsis and its alliance genera with the mechanisms largely remained unknown. Meiotic chromosome behavior of the pollen mother cells (PMC) was investigated to examine whether it is correlated with seed set in different breeding parents of both Doritaenopsis and Phalaenopsis orchids. The results showed that high percentage of normal tetrad, in addition to regular bivalent chromosome paring, generally suggests high fertility. Unreduced gametes such as dyads were observed during the PMC meiotic process and the number of the dyads could be increased by treating young flower buds with mitotic inhibitors such as colchicines and trifluralin. Through cross pollination of chemically conditioned pollinia, chance of obtaining polyploidy seedlings is expected to be increased.

Through these meiotic analysis and chemical treatment, breeding efficiency of phalaenopsis orchids will be increased.

Introduction

More than 50 species of *Phalaenopsis* genus have been found in the South-East Asia regions, including Taiwan, Philippine, Indonesia, Malaysia, Hainan Island, New Guinea, Thailand, and China (Christenson, 2001; Sweet, 1980). Its alliance genera such as *Doritis* and *Kingidium*, also have been used to produce the integeneric hybrids (http://www.phals.net/Definitions_e.html), especially *Doritaenopsis*. Many new *Phalaenopsis* and *Doritaenopsis* hybrids every year are registered through RHS (http://apps.rhs.org.uk/horticulturaldatabase/orchidregister/#). Potted Phalaenopsis orchids have become the most important flowering potted plants in the global markets (Griesbach, 2002). Therefore, breeding and selection of novel hybrids are the utmost to explore the Phalaenopsis market. To this point, how we can increase breeding efficiency by overcoming hybridization barriers, which occur very often during pollination and fertilization, is very helpful to the orchid industry.

Hybridization barriers in higher plants

The first step of fertilization in plants is pollination. Pollen tubes are allowed to grow into the stylar tissues in the campatible pollination. Increase in detectable levels of calcium ion was observed in the stylar tissues of lily after successful pollination and in the synergid cells within embryo sac of *Brassica napus* two days after fertilization (Zhao et al., 2004; Yu et al., 1998), which suggest an important role played by calcium in the process of reproduction (Ge et al., 2007). By monitoring Ca^{2+} dynamics during pollination in the transgenic *Arabidopsis* expressing yellow cameleon, a Ca^{2+} indicator based on green fluorescent protein, it was shown that

calcium was increased during pollen hydration and pollen tube penetrated into the papilla cell wall (Iwano et al., 2004). Some chemical guidance molecules, such as pectin and stigma/stylar cysteine-rich adhesin (SCA) have also been reported in the compatible pollination (Lord, 2003). Other molecules such as sugars and peptides may also help to guide pollen tube growth (Weterings and Russell, 2004). High levels of free auxin in stigmas and styles of *Nicotiana tabacum* during pollen germination and pollen-tube growth were detected by immunolocalization, which suggest the dependent role of auxin in pollination and subsequent fertilization (Chen and Zhao, 2008; Sundberg and Ostergaard, 2009). Certain compounds, including lipids, in the stigmatic exudates may help successful pollen tube germination (Sanchez et al., 2004).

In higher plants, two aspects of interspecific hybridization barriers are observed, including prezygotic and postzygotic barriers which may occur during or after syngamy (Stebbins, 1958; Rieseberg and Carney, 1998; Tiffin et al., 2001; Bushell et al., 2003). Imprinting and epigenetic regulation in interspecific hybrid failure have been suggested to operate in Arabidopsis, in addition to ploidy differences (Bomblies and Weigel, 2010).

One solution to the interspecific crossing barrier is to rescue the immature embryo by in vitro culture (Ondřej et al., 2001), or through in vitro fertilization (Wang et al., 2006). Alternatively, doubling the chromosome number by colchicines treatment of in vitro explants may help homeologous pairing in the doubled plants and thus improve the fertility (Hermsen et al., 1981; Lu and Bridgen, 1997).

Meiotic chromosome behavior and fertility in Doritaenopsis and Phalaenopsis orchids

Modern Phalaenopsis hybrids are usually derived from the cross hybridization between two different species or between a hybrid and a species. According to pedigree analysis by using the 'Wildcatt' software, one hybrid might be contributed by as many as 13-14 species via stepwise introgression. In addition, many hybrids have been evolved with higher ploidy level, especially tetraploid (Kuo et al., 2005). Most interspecific hybrids or intergeneric hybrid may carry diploidy. In dendrobium, the species from different sections may be represented as different genome constitution, therefore intersectional crossing might have abnormal meiotic pairing due to their nonhomologous chromosomes (Kamemoto et al., 1999). The chromosome set can be doubled by in vitro induction of mitotic inhibitors such as colchicines, oryzaline and some herbicides. With doubled chromosomes, the fertility of a hybrid may be restored completely or partially (van Tuyl et al., 1992; Lu and Bridgen, 1997).

Only limited information on breeding barrier of Phalaenopsis is available. In our experience, the chance of failure in capsule development in many crossing for either Doritaenopsis or Phalaenopsis orchids was high in practical experience. Crossing of diploid species *P*. *equestris* as female parent to tetraploid hybrids usually failed to set seeds. However, if the diploid species was used as pollen donor, 50-57% of the crosses produced viable seeds (Tang and Chen, 2007). When the triploid *P*. Taipei Gold 'STM' plant was crossed reciprocally to a tetraploid *P*. Sogo Yukidian 'V3', with regular meiosis (Hsu et al., 2010), no capsule or seed set was obtained. The cause for this crossing failure was attributed to the nature of low fertility in triploid as well as problem in stigmatic cavity clousure (Chuang et al., 2008). Despite auxins (IBA and NAA) were applied on to the stigma after pollination and cavity closed, no pollen germination was observed in the same cross combination (Chuang et al., 2008).

As the mechanisms of hybridization barriers in Phalaenopsis orchids are largely unknown, an alternative to look for the clue was by investigating the meiotic chromosome behavior of pollen mother cells (Bolaños-Villegas and Chen, 2007; Bolaños-Villegas et al., 2008), which may help to reveal suitable cultivars with good chromosome pairing and high pollen viability for use in the breeding program. The reason of using PMC as target material is their easy accessibility, because if we examine female meiosis of Phalaenopsis orchids, the fertilization event would occur only 60 days or 11 weeks after pollination and not very convenient for study (Nadeau et al., 1996; Lee et al., 2008).

Several Doritaenopsis hybrids were investigated for cross pollination, pollen viability, and chromosomal behavior during meiosis. A positive relation was found among high seed set, high frequency of viable tetrads, high degree of chromosome pairing, and low frequency of chromosomal aberrations such as inversions and translocations (Bolaños-Villegas and Chen, 2007; Bolaños-Villegas et al., 2008). In other words, those hybrids with lower percentage of tetrads, higher numbers of micronuclei or multivalent chromosome pairing tends to have low fertility, especially for the early generation *Doritaenopsis* hybrids such as *Dtps*. I-Hsin Purple Jewel, *Dtps*. Taihort Gem and *Dtps*. Queen Beer (Bolaños-Villegas and Chen, 2007; Bolaños-Villegas et al., 2008). The former two are also triploid with low or no fertility possibly due to abnormal meiotic behavior and low rate of normal tetrads. Generally, even numbered ploidy hybrids tend to have higher rate of tetrads and thus possessing certain degree of fertility. Triploids are generally sterile and only in some cases partially fertile which were supposed to be involved in polyploidy evolution (Bretagnolle and Thompson, 1995).

We further investigated some harlequin-type and standard pink Doritaenopsis for their meiosis, a high percentage of tetrads was observed in the PMCs of most hybrids

and thus their high fertility. One exception is with Dtps. Sogo Sofei, which had low fertility in several reciprocal crossing despite of its high tetrad percentage (Hsu et al., 2010).

Possibility of inducing unreduced gametes in Phalaenosis orchids

As mentioned above, meiotic pairing might be abnormal for *Dendrobium* species of different sections. In some cases, chromosome set were doubled between two different genomes and resulted with allopolyploid or amphidiploid. In many plants, such as Dendrobium orchid and a cactus, unreduced male or female gametes, occurring at very low rate, may contribute to produce polyploidy progeny (Kamemoto et al., 1999; Tel-Zur et al., 2003). Occasionally we could observe a tetraploid Phalaenopsis seedling derived from diploid plant crossed with another diploid, such as a tetraploid *P. equestris* was obtained and its ploidy level confirmed by root tip chromosome squash (Kuo et al., 2005). We suggest natural unreduced gamete as a good source for producing polyploidy progeny after cross pollination, despite with very low frequency.

To increase the number of unreduced gametes, mitotic inhibitors such as colchicine, oryzalin, and trifluralin have been used to prior to meiosis. Crossing plants with more unreduced gametes will increase the chance of polyploidy progenies (Bretagnolle and Thompson, 1995). We have used two tetraploid hybrids, namely *P.* Sogo Yukidian ‘V3’ and *P.* Tai Lin Redangel ‘V31’, as model phalaenopsis orchids to induce unreduced gametes. The reason for choosing both hybrids is their large flowers and pollinia for easy chemical treatment. Young flower buds were surface smeared with lanolin paste containing either colchicine or trifluralin. Both mitotic inhibitors increased the frequency of dyads in V3, while colchicines could induce higher number of unreduced dyads in V31 (Hsu et al., 2010).

Conclusion and perspectives

Meiotic chromosome behaviors of PMCs could be used as criteria for evaluation of parental fertility. They also revealed the state of unreduced gametes (monad, dyads and triads) and thus offer the opportunity to be induced at higher frequency by mitotic inhibitor treatments. Doubling the chromosome sets also may improve fertility. As yet we are too far from understating in depth of the hybridization barriers, those chemicals or metabolites which may help increase the pollination success and capsule setting so to overcome the barriers deserve further exploitation. Understanding hybridization barriers at molecular level may also shed light on the signals regulating the successful pollination and subsequent fertilization.

Literature cited

Bolaños-Villegas, P. and F. C. Chen. 2007. Cytological identification of chromosomal rearrangements in Doritaenopsis and Phalaenopsis. J. Intl. Coop. 2(1): 1-11

Bolaños-Villegas, P., S. W. Chin and F. C. Chen 2008. Meiotic chromosome behavior and capsule setting in *Doritaenopsis* hybrids. J. Amer. Soc. Hort. Sci. 133: 107-116.

Bomblies, K. and D. Weigel, 2010. Arabidopsis and relatives as models for the study of genetic and genomic incompatibilities. Phil. Trans. R. Soc. B 365: 1815-1823.

Bretagnolle, F. and J. D. Thompson. 1995. Gametes with the somatic chromosome

number: mechanisms of their formation and role in the evolution of autopolyploid plants. New Phytol. 129: 1-22.

Bushell, C., M. Spielman and R. J. Scott. 2003. The basis of natural and artificial postzygotic hybridization barriers in *Arabidopsis* species. Plant Cell 15: 1430-1442.

Chen, D. and J. Zhao. 2008. Free IAA in stigmas and styles during pollen germination and pollen tube growth of *Nicotiana tabacum*. Physiol. Plant. 134: 202-215.

Christenson, E. A. 2001. Phalaenopsios: A Monograph, Timber Press, Portland, Oregon.

Chuang, H. T., S. T. Hsu and T. M. Shen. 2008. Breeding barriers in yellow phalaenopsis orchids. J. Taiwan Soc. Hort. Sci. 54: 59-66.

Coyne, J. A. and H. A. Orr. 1998. The evolutionary genetics of speciation. Phil. Trans. R. Soc. Lond. B 353: 287-305.

Ge, L. L., H. Q. Tian and S. D. Russell. 2007. Calcium function and distribution during fertilization in angiosperms. Am. J. Bot. 94(6): 1046-1060.

Griesbach, R. J. 2002. Development of Phalaenopsis orchids for the mass-market. pp. 458-465. In: Janick, J. and A. Whipkey (eds.), Trends in New Crops and New Uses. ASHS Press, Alexandria, VA.

Hermsen, J. G. T. H., M. S. Ramanna and Z. Sawor. 1981. The effect of chromosome doubling on fertility, meiotic behaviour and crossability of *Solanum etuberosum* × *S. pinnatisectum*. Euphytica 30: 33-39.

Hsu, S. C., T. C. Cheng, P. Bolaños-Villegas, S. W. Chin and F. C. Chen. 2010. Pollen meiotic behavior in relation to *Phalaenopsis* breeding. Acta Hortic. (in press)

Iwano, M., H. Shiba, T. Miwa, F. S. Che, S. Takayama, T. Nagai, A. Miyawaki and A. Isogai 2004. Ca^{2+} dynamics in a pollen grain and papilla cell during pollination of *Arabidopsis*. Plant Physiol. 136: 3562-3571.

Kamemoto, H., T. D. Amore and A. R. Kuehnle. 1999. Breeding *Dendrobium* orchids in Hawaii. Univ. Hawaii Press, Honolulu.

Kuo, H. L., C. Y. Ke, Y. S. Chen, S. W. Chin and F. C. Chen. 2005. Study on root tip squash and staining techniques of *Phalaenopsis* orchids. J. Chinese Soc. Hort. Sci. 51: 339-345.

Lee, Y. -I., E. C. Yeung, N. Lee and M. -C. Chung. 2008. Embryology of *Phalaenopsis amabilis* var. *formosa*: embryo development. Bot. Stud. 49: 139-146.

Lord, E. M. 2003. Adhesion and guidance in compatible pollination. J. Exp. Bot. 54: 47-54.

Lu, C. and M. P. Bridgen. 1997. Chromosome doubling and fertility study of *Alstroemeria aurea* × *A*. *caryophyllaea*. Euphytica 94: 75-81.

Nadeau, J. A., X. S. Zhang, J. Li and S. D. O'Neill. 1996. Ovule development: Identification of stage-specific and tissue-specific cDNAs. Plant Cell 8: 213-239.

Ondřej, V., B. Navrátilová and A. Lebeda. 2001. Determination of the crossing barriers in hybridization of *Cucumis sativus* and *Cucumis melo*. Cucurbit Genet. Coop. Rep. 24: 1-5.

Rieseberg, L. H. and S. E. Carney. 1998. Plant hybridization. New Phytol. 140: 599-624.

Sanchez, A. M., M. Bosch, M. Bots, J. Nieuwland, R. Feron and C. Mariani. 2004. Pistil Factors Controlling Pollination. Plant Cell 16(suppl_1): S98-S106.

Stebbins, G. L. 1958. The inviability, weakness and sterility of interspecific hybrids. Adv. Genet. 9: 147-215.

Sundberg, E. and L. Ostergaard. 2009. Distinct and dynamic auxin activities during reproductive development. Cold Spring Harb. Perspect. Biol. 1(6): a001628-a001628.

Sweet, H. R. 1980. The genus *Phalaenopsis*. Day Printing Corp., Pomona, California.

Tang, C. Y. and W. -H. Chen. 2007. Breeding and development of new varieties in Phalaenopsis. In: Chen, W. -H. and H. -H. Chen (eds), Orchid Biotechnology, Chapter 1, pp. 1-22. World Sci. Pub., Singapore.

Tel-Zur, N., S. Abbo, D. Bar-Zvi and Y. Mizrahi. 2003. Chromosome doubling in vine cacti hybrids. J. Hered. 94: 329-333.

Tiffin, P., M. S. Olson and L. C. Moyle. 2001. Asymmetrical crossing barriers in angiosperms. Proc. R. Soc. Lond. B Biol. Sci. 268: 861-867.

Van Tuyl, J. M., B. Meijer and M. P. Van Diën. 1992. The use of oryzalin as an alternative for colchicines in *in vitro* chromosome doubling of *Lilium* and *Nerine*. Acta Hortic. 325: 625-630.

Wang, Y. Y., A. Kuang, S. D. Russell and H. Q. Tian. 2006. In vitro fertilization as a tool for investigating sexual reproduction of angiosperms. Sex. Plant Reprod. 19: 103-115..

Weterings, K. and S. D. Russell. 2004. Experimental analysis of the fertilization process. Plant Cell 16: S107-S118.

Yu, F. -L., S. -P. Liang, H. -Y. Yang and Y. Wang. 1998. Ultracytochemical localization of calcium in micropyle and embryo sac of *Brassica napus* before and after pollination. Acta Bot. Sinica 40: 591-597.

Zhao, J., H. -Y. Yang and E. M. Lord. 2004. Calcium levels increase in the lily stylar transmitting tract after pollination. Sex. Plant Reprod. 16: 259-263.

Growth and physiological responses of ornamental plants to salinity

Genhua Niu

Texas AgriLife Research and Extension Center at El Paso, Texas A&M System, 1380 A&M Circle, El Paso, TX 79927, USA

Author for correspondence : gniu@ag.tamu.edu

Abstract

Water shortage and poor water quality are global issues. With rapid population growth and the diminishing water supply in urban areas, use of alternative water sources such as municipal recycled water and other sources of non potable waters for agriculture and landscape irrigation is inevitable. A potential concern is the elevated salt levels in these alternative waters. This paper briefly summarizes our research results on salt tolerance of a large number of ornamental plants ranging from bedding plants, herbaceous perennial and groundcovers and woody shrubs and trees. The methodology of screening and evaluating salt tolerance of ornamental plants is discussed. Like agricultural crops, a wide range of salt tolerance among ornamental plants has been found. In addition to plant species, dominant salt type, substrate, irrigation method and management, and environmental conditions also affect plant responses to salinity stress. A number of mechanisms of salinity tolerance have been observed among these ornamental species, including restriction of ion uptake, selective ion uptake, and tolerance to high internal concentrations of sodium and/or chloride.

Introduction

Ornamental crops are traditionally irrigated with high-quality water due to their high cash values and most of them were considered sensitive to salinity. With the increasing population and industrial development, high quality fresh water is becoming a limited and expensive resource in many regions of the world. Agriculture, including ornamental industry, has to compete with industrial and domestic water uses. It is time to explore alternative water sources such as municipal recycled water and brackish groundwater for agricultural irrigation. The municipal recycled water (also called "reclaimed water") is the only water source that increases with population growth (Qian et al, 2005). In the U.S. (Dobrowolski et al., 2008; Morgan et al., 2008) and other countries (Pasternak and Malach, 1994; Safi et al., 2007; Tanwar, 2003), the municipal recycled water has already been used for irrigating urban landscapes and field crops for decades where potable water supply is limited. The potential problem for using these alternative water sources is the high salt levels, which causes salt damage or even death on sensitive plants, if not managed properly.

Soil salinity is already a problem in arid and semi-arid areas where irrigation is practiced. Over 50% of all irrigated lands are affected by salinization, yet the water used for irrigating these lands was seldom saline (Pasternak and Malach, 1994). Soil salinization would become even worse with more and more alternative or low quality water is being or will be used for irrigation. In addition, continuous sea-level rise in a warming world threatens increased salinity in coastal lowlands (Rozema and Flowers, 2008). As fertile soils become salinized, the yield of conventional crops decreases (Pasternak and Malach, 1994).

One of the common solutions to overcome salinity problems is the introduction of salt tolerant crops. Like agronomic crops, salt tolerance of ornamental plants varies largely with species and even cultivars within a species. Therefore, screening and

evaluating the salt tolerance of commercially important ornamental plants are imperative. Such evaluations may facilitate improvement of salt tolerance of ornamental plants in breeding and bioengineering programs or prove the feasibility of irrigating with saline water for salt tolerant ornamental plants. This paper discusses the methodology of salt tolerance evaluation and summarizes the research results on salt tolerance of ornamental plants conducted in the past few years in our laboratory.

Methods of evaluating salt tolerance for ornamental plants

Greenhouse studies. Compared to field trials, greenhouse studies for screening salt tolerance are less labor intensive, less costly, and the environmental conditions can be more easily controlled. Salt treatments in greenhouse studies were created by adding salts to irrigation water (tap water or nutrient solution) to mimic a composition of a target saline water source or saline soil. A large number of salinity studies have used NaCl as the sole salinizing agent (Cassaniti et al., 2009; Eom et al., 2007; Marosz, 2004; Fostad and Pedersen, 2000; Wahome, 2003; Wu et al., 2001). However, composition of salts in water and salt-affected soils varies throughout the world (Carter and Grieve, 2006; Grattan and Grieve, 1999). Saline solutions with a single salt may result in misleading and erroneous interpretations about plant response to salinity (Carter and Grieve, 2006; Grattan and Grieve, 1999). In our studies, NaCl + $CaCl_2$ + $MgSO_4$ (87:8:5 by weight) have been added to tap water or nutrient solutions to mimic the major ion composition of local reclaimed waters. A wide range of salinity levels, depending on the estimated salt tolerance of the species to be tested, are prepared to distinguish the differences in plant response to increasing salinity. Even when salinity levels of irrigation water are kept the same, the maintenance of constant root zone salinity levels is difficult, and equally challenging is its measurement because of the strong influence of soil moisture content (Marosz, 2004; Tanji, 2002). The concentration of dissolved salts does not change in direct

proportion to changes in soil moisture content due to solubility, cation exchange and ion association issues.

Salinity is a dynamic property in the rootzone, due to evapo-concentration of the soil solution, water extraction, selective plant uptake from plant roots, and replenishment by irrigation or rainfall (Tanji, 2002). Salt accumulation in substrates is also influenced by plant taxa and genotypes, substrate physical-chemical properties, plant water use, irrigation frequency, and drainage and/or leaching fraction (Bernstein et al., 2006; Niu and Rodriguez, 2006a, b). To prevent salt accumulation, increasing leaching fraction and using porous, well drained substrates are effective approaches. However, high leaching fraction leads to low irrigation efficiencies (Niu et al., 2007b).

Outdoor container and field studies. Environmental conditions such as temperature, light intensity, humidity, and wind speed can considerably affect plant response to salinity (Fox et al., 2005; Niu et al., 2007a; Zollinger et al., 2007). Therefore, salt tolerance of plants from greenhouse studies provides a relative order of salt tolerance among tested plant species. The absolute salt tolerance in outdoor conditions for ornamental plants can be determined on limited number of plants with known salt tolerance under greenhouse environment. For field studies, soils will be salinized after a salt tolerance study, and thus will require replacement prior to re-utilization in subsequent experiments in the same field, unless all accumulated salts can be leached out. In our research, raised beds have been used and soils in the beds are replaced prior to each experiment (Niu et al., 2007a). Treatments were created similarly to greenhouse studies by adding salts at various ratios to irrigation water to create various salinity levels. Like greenhouse container studies, salt accumulation in the field varied among beds and locations within the same bed due to differences in water use of the plants and micro-environment. Outdoor studies using containers can provide outdoor environmental conditions, while eliminating the variation among

adjacent plots and easing the control and disposal of salinized substrates or soils. In reality, selection of method for screening salt tolerance often depends on target plant species, seasons, and availability of resources.

General responses of ornamental plants to salinity

Salt tolerance of a plant is defined as the ability to withstand the effects of high salinity without significant adverse effects such as growth or yield reduction, or foliar salt damage (Grieve et al., 2008). Salinity reduces the ability of plants to take up water, and this quickly causes reductions in growth rate, along with a suite of metabolic changes identical to those caused by water stress (Marschner, 1995; Munns, 2002). For ornamental plants, the typical symptoms of initial salt injury are stunted growth and foliar damage including leaf necrosis, and marginal leaf burn (Bernstein et al., 1972). As salt stress becomes severe, leaves are generally negatively impacted, resulting in fewer green leaves. For flowering woody shrubs and trees, buds may fail to open or grow and branches may die in salt-stressed shrubs and trees (Azza Mazher et al., 2007; Niu and Rodriguez, 2007). Salt injury symptoms are more evident under hot, dry and windy conditions than in a cool, humid environment (Fox et al., 2005; Niu et al., 2007a; Wu et al., 2001). Salt tolerance of a plant varies largely with species or even cultivar within a species (Cabrera, 2009; Niu et al., 2007a, c). The actual response of a plant to salinity is often affected by climatic conditions, type of substrate or soil, irrigation management, and plant growth stage. For each salinity level, it is possible to select a number of ornamental plants whose aesthetic values are not or only slightly affected (Bernstein et al., 1972). Obviously, as salinity levels increase, the number of plants that can tolerate the salt stress becomes smaller.

High salinity induces a series of metabolic dysfunctions in ornamental plants, including absorption of excessive minerals, nutrient imbalance, photosynthesis

inhibition, enzymatic activity, protein and nucleic metabolism and respiration (Azza Mazher et al., 2007; Marschner, 1995; Munns and Tester, 2008; Niu and Rodriguez, 2006a, b; Niu et al., 2008; Niu and Rodriguez, 2008). The extent of adverse impact of salinity on the above physiological processes depends on salinity levels and exposure period.

Growth and aesthetic responses of ornamental plants to salinity

Depending on species or genotype, growth can be quantified non-destructively by recording plant height and/or canopy size periodically. All parts of a plant, including leaves, stems, roots, and fruits may be reduced in size under saline conditions. Usually, growth reduction in shoots is greater than roots (Munns, 2002; Niu and Rodriguez, 2006a, b; Shannon et al., 1994). For agricultural crop plants, salt tolerance is often assessed based on growth or yield reduction of specific plant parts such as seeds, roots, fruits, or leaves (Maas, 1990; Pasternak and De Malach, 1994). However, for ornamental plants, aesthetic value is more important than growth rate (Bernstein et al., 1972). Therefore, visual quality, although it can be highly subjective, is an important parameter for assessing salt tolerance of ornamental plants (Devitt et al., 2005; Fox et al., 2005; Niu and Rodriguez, 2006a, b; Niu et al., 2007a; Zollinger et al., 2007). Dry matter accumulation and/or relative growth rate are commonly used to evaluate salt tolerance of plants, but this parameter is not available until plants are harvested destructively. The timing when significant differences in foliar damage symptoms appear is another parameter used for the assessment of plant salt tolerance. The earlier the salt injury is exhibited in a plant, the less is its tolerance to salinity. For a quick reference, Tables 1, 2, and 3 list relative salinity tolerances of ornamental plants evaluated based on visual salt injury and growth reduction.

Physiological responses of ornamental plants to salinity

In addition to these visible changes reflected in plant growth/size and foliar damage, plants also respond to salinity internally, expressed in changes in physiological and metabolic processes. To quantify the physiological responses to salinity, photosynthesis, leaf conductance, chlorophyll fluorescence, chlorophyll content, ion uptake and accumulation in tissues are typically measured (Cabrera, 2003; Eom et al., 2007; Niu and Rodriguez, 2006a; Quist and Williams, 1999; Wu et al., 2001; Zollinger et al., 2007; Zribi et al., 2009).

Osmotic adjustment. Osmotic adjustment occurs in plants subjected to salinity stress by accumulation of high concentrations of either inorganic ions or organic solutes (or both) (Ashraf, 2004). In our studies, we found that leaf osmotic potential of herbaceous perennials and bedding plants generally decreases with increasing salinity of irrigation water (Niu and Rodriguez, 2006a, b; Niu et al., 2010b). There were interactions between salinity and species on osmotic potentials of the tested species, indicating that the effect of salinity on osmotic adjustment varied with genotypes/species. For example, among selected bedding plants (Niu et al., 2010b), osmotic potentials of angelonia 'Purple', ornamental pepper 'Black Pearl' and 'Calico', helenium, vinca, and licorice plants decreased linearly as salinity of irrigation water increased. In angelonia 'Lavender Pink' and ornamental pepper 'Purple Flash', salinity treatment did not affect leaf osmotic potentials. In angelonia 'White' and plumbago, osmotic potential followed a quadratic fashion as salinity of irrigation water increased.

Ion uptake. Plant adaptations to salinity are of three distinct types: osmotic stress tolerance, Na or Cl exclusion, and the tolerance of tissue to accumulated Na or Cl (Munns and Tester, 2008). Salt tolerant genotypes usually have the ability to restrict Na or Cl transport to shoots. Some tolerant genotypes can tolerate high internal Na or

Cl concentrations. Ion uptake depends on species or genotype, salinity level, and the chemical composition of the soil solution (Grattan and Grieve, 1999; Niu and Rodriguez, 2008).

Niu and Rodriguez (2006a) studied differences in overall salt tolerance and Na or Cl tolerance among eight herbaceous species irrigated with saline solutions at four salinity levels. Four species died by the middle of the 12-week experiment. Among the surviving four species, the ion accumulation in shoots and roots differed. Both Na and Cl in shoots and roots generally increased with salinity of irrigation water in all species but were different among species. For example, *Gazania rigen* had the highest Na concentration in shoots (39 $mg \cdot g^{-1}$ DW) but *Delosperma cooperi* had the highest Na concentration in roots (22 $mg \cdot g^{-1}$ DW). Shoot Cl was highest in *D. cooperi* and *G. rigen*, and even in the control plants. The Cl concentration was 5 to 10 times higher in *D. cooperi* and *G. rigen* than the other two species in all treatments. Regardless of the high Na or Cl concentrations, no visual salt injury was observed in *G. rigen* and *D. cooperi*, indicating that these two species were tolerant to high internal Na or Cl concentrations. Compared to *G. rigen*, *D. cooperi* had a higher ability to exclude Na from shoots, which is an important mechanism of salt tolerance.

Salt composition affects the response of ornamental plants to salinity. Growth and ion uptake of four rose rootstocks responded to salinity and dominant salt type differently (Niu and Rodriguez, 2008). *Rosa ×fortuniana* was the most tolerant rootstock when irrigated with a saline solution dominated by sulfate, followed by *R.* 'Dr. Huey', *R. multiflora*, and *R. odorata* in descending order. However, *R. ×fortuniana* was the least tolerant rootstock when chloride was dominant in the saline solutions, *R. odorata* was the second least tolerant, and 'Dr. Huey' was the most tolerant. *Rosa ×fortuniana* and 'Dr. Huey' had a higher restriction ability of Na uptake than the other two rootstocks.

Leaf gas exchange. The most dramatic and readily measurable whole plant response to salinity is a decrease in stomatal aperture (Munns and Tester, 2008). Rates of photosynthesis per unit leaf area in salt-treated plants are often unchanged, even though stomatal conductance is reduced (James et al., 2002). Net photosynthetic rate of four rose rootstocks (*Rosa ×fortuniana, R. multiflora, R. odorata* and *R. hybrida* 'Dr. Huey'), which were irrigated with saline solutions up to 8.0 $dS \cdot m^{-1}$, were not affected (Niu, unpublished data), possibly because the healthy, young leaves were selected. The effect of salinity on photosynthesis and stomatal conductance depends on species and salinity level. For example, *Ziziphus nummularia* had greater reduction in photosynthesis and stomatal conductance than *Z. rotundifolia* (Meena et al., 2003). For the salt sensitive green ash (*Fraxinus pennsylvanica*), both stomatal conductance and net photosynthesis decreased significantly when seedlings were subjected to salt stress ranging from seawater concentration to one-tenth the concentration of seawater (Pezeshki and Chambers, 1986). Conversely, for the halophyte *Salvadora persica*, net photosynthesis, stomatal conductance and transpiration rate were not reduced by salinity stress at 200 mM NaCl compared to the control without NaCl (Maggio et al., 2000). Net photosynthesis, stomatal conductance and transpiration rates of *Phillyrea latifolia* grown at various salinity concentrations markedly decreased and the differences of these rates between the control and salinity treatments became larger over time (Tattini et al., 2002). Summarizing the above, the effect of salinity on leaf gas exchange depends on many factors such as leaf age, the level of salinity, and duration of salinity stress exposure, in addition to species and genotype.

Chlorophyll fluorescence. Chlorophyll fluorescence has been proved to be useful in salinity tolerance evaluation. The maximal photochemical efficiency of photosystem II (PSII), ratio of variable fluorescence (F_v) to maximal fluorescence (F_m), F_v/F_m, is commonly measured to examine damage in the photosynthetic

apparatus caused by salinity stress (Jimenez et al., 1997; Percival, 2005; Percival and Fraser, 2001; Sixto et al., 2006). Percival (2005) concluded that measuring chlorophyll fluorescence (F_v/F_m) of excised, salt-treated leaves under laboratory conditions can be used to evaluate salt tolerance without destroying whole plants. In our studies, we found that F_v/F_m was reduced by elevated salinities at 3.0 and 6.0 $dS \cdot m^{-1}$ in three rose rootstocks, but the reduction was small (Niu et al., 2008). The advantages of using chlorophyll fluorescence include the fact that fluorescence measurements use a light portable piece of equipment, and measurements are non-destructive and non-invasive. The effectiveness depends on species and salinity levels. For example, no differences were found in F_v/F_m in tomato leaves treated with salinity up to 200 mM NaCl (equivalent to18.3 $dS \cdot m^{-1}$) in dark-adapted leaves (Zribi et al., 2009). However, the PSII photochemistry was modified in salt-affected plants in light-adapted leaves. Naumann et al. (2008) also reported that significant differences in light-adapted fluorescence yield were observed by day 3 in *Myrica cerifera* plants treated with salinity stress while the dark-adapted fluorescence yield did not show differences between the salinity treatment and control until day 12, which was well after visible signs (day 5) of stress were apparent.

Summary

Salt tolerance of ornamental plants varies widely with species, environmental conditions, and soil or substrate. Ornamental plants, most of which are non-halophyte, have similar mechanisms of salt tolerance to agricultural crops including osmotic adjustment, Na exclusion, selective uptake of ions, and tolerance of high international accumulated Na^+ and/or Cl^-. Elevated salinity in irrigation water reduces plant size and growth, causes foliar injury (reducing aesthetic value), and increases soil salinity. A wide range of tolerance to salinity exists among tested ornamental plants.

Assessment of salt tolerance for ornamental plants should be based primary on aesthetic value.

Literature cited

Ashraf, M. 2004. Some important physiological selection criteria for salt tolerance in plants. Flora 199: 361-376.

Azza Mazher, A. M., E. M. Fatma El-Questni, and M. M. Farahat. 2007. Responses of ornamental plants and woody trees to salinity. World J. Ag. Sci. 3: 386-395.

Bernstein, L., L. E. Francois and R. A. Clark. 1972. Salt tolerance of ornamental shrubs and ground covers. J. Amer. Soc. Hort. Sci. 97: 550-556.

Bernstein, N., B. T. Asher, F. Haya, S. Pini, R. Ilona, C. Amram, and I. Marina. 2006. Application of treated wastewater for cultivation of roses (*Rosa hybrida*) in soil-less culture. Scientia Hort. 108: 185-193.

Boland, A. M. 2008. Management of saline and/or recycled water for irrigated horticulture. Acta Hort. 792: 123-134.

Cabrera, R. I. 2003. Growth, quality and nutrient responses of azalea hybrids to salinity. Acta Hort. 609: 241-245.

Cabrera, R. I. 2009. Revisiting the salt tolerance of crape myrtles (*Lagerstroemia* spp.). Arboric. Urban Fores. 35(3): 129-34.

Carter, C. T. and C. M. Grieve. 2006. Salt tolerance of floriculture crops, p. 279-287. In: M. A. Khan and D. J. Weber (eds.). Ecophysiology of high salinity tolerant plants. Springer, Netherlands.

Cassaniti, C., C. Leonardi, and T. J. Flowers. 2009. The effects of sodium chloride on ornamental shrubs. Scientia Hort. 122: 586-593.

Devitt, D. A., R. L. Morris, and L. K. Fenstermaker. 2005. Foliar damage, spectral reflectance, and tissue ion concentrations of trees sprinkle irrigated with waters of similar salinity but different chemical composition. HortScience 40: 819-826.

Dobrowolski, J., M. O'Neill, L. Duriancik, and J. Throwe (eds.). 2008. Opportunities and challenges in agricultural water reuse: Final report. USDA-CSREES, 89 p.

Eom, S. H., T. L. Setter, A. DiTommaso, and L. A. Weston. Differential growth response to salt stress among selected ornamentals. J. Plant Nutri. 30: 1109-1126.

Fostad, O. and P. A. Pedersen. 2000. Container-grown tree seedling responses to sodium chloride applications in different substrates. Environmental Pollution 109: 203-210.

Farnham, D. S., R. F. Hasek and J.L. Paul. 1985. Water quality: Its effects on ornamental plants. University of California Cooperative Extension Leaflet No. 2995, 15 p.

Fox, L. J., J. N. Grose, B. L. Appleton, and S. J. Donohue. 2005. Evaluation of treated effluent as an irrigation source for landscape plants. J. Env. Hort. 23: 174-178.

Grattan, S. R. and C. M. Grieve. 1999. Salinity-mineral nutrient relations in horticultural crops. Scientia Hort. 78: 127-157.

Grieve, C., L. Wu, L. Rollins, and A. Harivandi. 2008. Tolerance by landscape plants of salinity and of specific ions (Chapter V). In: A comprehensive literature review on salt management guide for landscape irrigation with recycled water in coastal southern California. www.salinitymanagement.org. 320 p.

Jimenez, M. S., A. M. Gonzalez-Rodriguez, D. Morales, M. C. Cid, A. R. Socorro, and M. Caballero. 1997. Evaluation of chlorophyll fluorescence as a tool for salt stress detection in roses. Photosynthetica 33(2): 291-301.

James, R. A, A. R. Rivelli, R. Munns, and S. von Caemmerer. 2002. Factors affecting CO_2 assimilation, leaf injury and growth in salt-stressed durum wheat. Funct. Plant Biol. 29: 1393–403.

Maas, E. V. 1990. Crop salt tolerance, p. 262-304. In: K.K. Tanji (ed.). Agricultural salinity assessment and management. Amer. Soc. of Civil Engineers, ASCE Manuals and reports on engineering practice No. 71. New York.

Maggio, A., M.P. Reddy, and R. J. Joly. 2000. Leaf gas exchange and solute accumulation in the halophyte *Salvadora persica* grown at moderate salinity. Environmental and Experimental Botany 44: 31-38.

Marosz, A. 2004. Effects of soil salinity on nutrient uptake, growth, and decorative value of four ground cover shrubs. J. Plant Nutrition 27: 977-989.

Marschner, H. 1995. Mineral nutrition of higher plants, 2nd Edition. Academic Press. San Diego, CA.

Meena, S. K, N. K. Gupta, S. Gupta, S. K. Khandelwal, and E. V. D. Sastry. 2003. Effect of sodium chloride on the growth and gas exchange of young Ziziphus seedling rootstocks. J. Hort. Sci. Biotech. 78: 454-457.

Morgan, K. T., T. A. Wheaton, L. R. Parsons, and W. S. Castle. 2008. Effects of reclaimed municipal waste water on horticultural characteristics, fruit quality, and soil and leaf mineral concentration of citrus. HortScience 43: 459-464.

Munns, R. 2002. Comparative physiology of salt and water stress. Plant Cell Env. 25: 239-250.

Munns, R. and M. Tester. 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 59: 651-681.

Naumann, J. C., D. R. Young, and J. E. Anderson. 2008. Leaf chlorophyll fluorescence, reflectance, and physiological response to freshwater and saltwater flooding in the evergreen shrub, *Myrica cerifera*. Environmental and Experimental Botany 63: 402-409.

Niu, G. and D. S. Rodriguez. 2006a. Relative salt tolerance of selected herbaceous perennials and groundcovers. Scientia Hort. 110: 352-358.

Niu, G. and D. S. Rodriguez. 2006b. Relative salt tolerance of five herbaceous perennials. HortScience 41: 1493-1497.

Niu, G. and D. S. Rodriguez. 2007. Salinity tolerance, water use and crop coefficients of selected shrubs and trees. Proc. Southern Nursery Association 52: 133-137.

Niu, G., D. S. Rodriguez, and L. Aguiniga. 2007a. Growth and landscape performance of ten herbaceous species in response to saline water irrigation. J. Env. Hort. 25: 204–210.

Niu, G., D. S. Rodriguez, and Y. T. Wang. 2007b. Salinity and growing medium regulate growth, morphology and ion uptake of *Gaillardia aristata*. J. Env. Hort. 25(2): 89-94.

Niu, G., D. S. Rodriguez, W. Mackay, and L. Aguiniga. 2007c. Salinity tolerance of *Lupinus havardii* and *L. texensis.* HortScience 42(3): 526-528

Niu, G. and D. S. Rodriguez. 2008. Responses of growth and ion uptake of four rose rootstocks to chloride- or sulfate-dominated salinity. J. Amer. Soc. Hort. Sci. 133(5): 663–669.

Niu, G., D. S. Rodriguez, and L. Aguiniga. 2008. Effect of saline water irrigation on growth and physiological responses of three rose rootstocks. HortScience 43(5): 1479–1484.

Niu, G., D. S. Rodriguez, and M. Gu. 2010a. Salinity tolerance of *Sophora secundiflora* and *Cercis canadensis var. Mexicana*. HortScience 45(3): 424-427.

Niu, G., D. S. Rodriguez, and T. Starman. 2010b. Response of bedding plants to saline water irrigation. HortScience 45(4): 628-636.

Pasternak, D. and Y. D. Malach. 1994. Crop irrigation with saline water. In: Pessarakli Mo (ed.), Handbook of Plant and Crop Stress. Marcel Dekker, New York, p. 599-622.

Percival, G. C. 2005. Identification of foliar salt tolerance of woody perennials using chlorophyll fluorescence. HortScience 40: 1892-1897.

Percival, G.C. and G.A. Fraser. 2001. Measurement of the salinity and freezing tolerance of Crataegus genotypes using chlorophyll fluorescence. J. Arbor. 27(5): 233-245.

Pezeshki, S. R. and J. L. Chambers. 1986. Effect of soil salinity on stomatal conductance and photosynthesis of green ash (*Fraxinus pennsylvanica*). Can. J. For. Res. 16:569-573.

Qian, Y. L., J. M. Fu, J. Klett, and S. E. Newman. 2005. Effects of long-term recycled wastewater irrigation on visual quality and ion concentrations of ponderosa pine. J. Environ. Hort. 23: 185-189.

Quist, T. M. and C. F. Williams. 1999. Irrigation water quality and ion balance in leaves of deciduous ornamental trees. J. Plant Nutri. 22(6): 1011-1019.

Rozema, J., Flowers, T., 2008. Crops for a Salinized World. Science 322, 1478-1480.

Safi, M. I., A.Fardous, M. Muddaber, S. Ei-Zuraiqi, A. Balaweneh, L. Al-Hadidi and I. Bashabsheh. 2007. Long term effects of reclaimed water on rose and carnation

cut flower crops in soil and soilless media. Journal of Applied Sciences 7: 1191-1198.

Shannon, M. C., C. M. Grieve, and L. E. Francois. 1994. Whole-plant response to salinity. In: R.E. Wilkinson (ed.), Plant Environment Interaction. Marcel Dekker, New York, p.199-244.

Sixto, H., I. Aranda, and J. M. Grau. 2006. Assessment of salt tolerance in Populus alba clones using chlorophyll fluorescence. Photsynthetica 44(2): 169-173.

Tanji, K. K. 2002. Salinity in the soil environment. In: A. Läuchli and U. Lüttge (eds.), Salinity: Environment - Plants – Molecules. Kluwer Academic Publishers, Netherlands, p. 21-51.

Tanwar, B. S. 2003. Saline Water Management for Irrigation (3rd revised draft), Work Team on Use of Poor Quality Water for Irrigation (WT-PQW), International Commission on Irrigation and Drainage (ICID), New Delhi, India.

Tattini, M., G. Montagni, and M. L. Traversi. 2002. Gas exchange, water relations and osmotic adjustment in Phillyrea latifolia grown at various salinity concentrations. Tree Physiology 22:403-412.

Wang, M., G. Niu, D. S. Rodriguez, and D. Zhang. 2010. Responses of Zinnia marylandica cultivars to salinity. Proc. Southern Nursery Association. (in Press)

Wahome, P. K. 2003. Mechanisms of salt (NaCl) stress tolerance in horticultural crops – a mini review. Acta Hort. 609: 127-131.

Wu, L., X. Guo, and A. Harivandi. 2001. Salt tolerance and salt accumulation of landscape plants irrigated by sprinkler and drip irrigation systems. J. Plant Nutr. 24: 1473-1490.

Zollinger, N., R. Koenig, T. Cerny-Koenig, and R. Kjelgren. 2007. Relative salinity tolerance of intermountain western United States native herbaceous perennials. HortScience 42: 529-534.

Zribi, L., G. Fatma, R. Fatma, R. Salwa, N. Hassan, and R. Mohamed Ne´ jib. 2009. Application of chlorophyll fluorescence for the diagnosis of salt stress in tomato (*Solanum lycopersicum*, variety Rio Grande). Scientia Hort. 120: 367-372.

Table 1. Salt tolerance of bedding plants (Niu et al., 2007c; Niu et al., 2010b; Wang et al., 2010).

Botanical name	Common name	Cultivar	Tolerance
Angelonia angustifolia	Angelonia	Lavender Pink	MT
A. angustifolia	Angelonia	Purple	MT
A. angustifolia	Angelonia	White [z]	MT
Capsicum annuum	Ornamental pepper	Black Pearl	M
C. annuum	Ornamental pepper	Calico	M
C. annuum	Ornamental pepper	Purple Flash	M
Catharanthus roseus	Vinca	Rose	M
Gomphrena sp.	Gomphrena	Fireworks	MT
Helenium amarum	Helenium	Dakota Gold	MT
Helichrysum petiolatum	Licorice Plant	Silver Mist	MT
Lupinus havardii	Big bend bluebonnet		M
L. texensis	Texas bluebonnet		MT
Petunia x *hybrida*	Petunia	Baby Duck Yellow	MT
P. x *hybrida*	Petunia	Mirage Rose	MT
P. x *hybrida*	Petunia	Spreading Rose	MT
Plumbago auriculata	Plumbago	Escapade Blue	MT
Zinnia marylandica	Zinnia	Zahara Coral Rose	S
Z. marylandica	Zinnia	Zahara Fire	S
Z. marylandica	Zinnia	Zahara Rose Starlight	S
Z. marylandica	Zinnia	Zahara Scarlet	S
Z. marylandica	Zinnia	Zahara White	S
Z. marylandica	Zinnia	Zahara Yellow	S
Z. maritime	Zinnia	Solcito	S

T: tolerant; plants plants did not have any visual salt injury with minimum growth reduction at electrical conductivity of irrigation water at 12 $dS \cdot m^{-1}$ or above.

MT: moderate tolerant; plants did not have any visual salt injury with minimum growth reduction at electrical conductivity of irrigation water at 4 to 6 $dS \cdot m^{-1}$ or above.

M: moderate tolerant; plants had minimum visual salt injury and acceptable growth reduction at electrical conductivity of irrigation water at 3-4 $dS \cdot m^{-1}$.

MS: moderate sensitive; plants exhibited salt injury and growth reduction at electrical conductivity of irrigation water at 3-4 $dS \cdot m^{-1}$ or above.

S: sensitive; plants did not surve or had severe salt injury and significant growth reduction at electrical conductivity of irrigation water at 3-4 $dS \cdot m^{-1}$ or above.

Table 2. Relative salt tolerance of herbaceous perennials and groundcovers (Niu and Rodriguez, 2006a, b; Niu et al., 2007a, b).

Botanical name	Common name	Tolerance
Achillea millefolium	Yarrow	M
Agastache cana	Wild hyssop	MS
Berlandiera lyrata	Chocolate daisy	S
Ceratostigma plumbaginoides	Hardy blue plumbago	M
Delosperma cooperi	Ice plant	T
Echinacea purpurea	Purple coneflower	S
Gaillardia aristata	Firewheel	MT
Gazania rigen	Gazania	MT
Lantana	New gold lantana	MT
L. camara	Purple lantana	M
Lavandula angustifolia	Lavender	MS
Lonicera haliana	Honeysuckle	MT
Penstemon eatonii	Firecracker penstemon	S
P. pseudospectabilis	Pink showy penstemon	S
P. strictus	Rocky mountain penstemon	S
Rosmarinus officinalis	Rosemary	MT
Rudbeckia hirta	Black-eyed susan	S
Salvia coccinea	Scarlet sage	S
Teucrium chamaedrys	Germender	M
Verbena canadensis	Purple verbena	M
V. hybrida	Garden verbena	M
V. macdougalii	Spike verbena	M

For tolerance symbols: T, MT, M, MS, S, refer to the footnote of Table 1.

Table 3. Relative salt tolerance of ornamental shrubs and trees (Niu and Rodriguez, 2007; Niu et al., 2010a).

Botanical name	Common name	Tolerance
Cercis canadensis var. Mexicana	Mexican redbud	S
Chilopsis linearis	Desert willow	MS
Elaeagnus angustifolia	Russian olive	MS
Fraxinus pennsylvanica	Green ash	MS
Prunus angustifolia	Sand plum	S
P. besseyi	Sand cherry	S
P. serotina	black cherry	S
Sophora secundiflora	Texas Mountain laurel	M
Ulmus parvifolia	Chinese elm	MT

For tolerance symbols: T, MT, M, MS, S, refer to the footnote of Table 1.

Temperature acclimation before shipping and light acclimation after shipping improved the performance of *Phalaenopsis* after prolonged dark shipping

Yao-Chien Alex Chang* and Jiunn-Yan Hou

Department of Horticulture, National Taiwan University, Taipei 10617, Taiwan

* Author for correspondence : alexchang@ntu.edu.tw

Abstract

Mature, ready-to-bloom *Phalaenopsis* plants are often shipped by sea, where they experience total darkness for a few weeks in cool air, in stark contrast to warm growing conditions in the greenhouse. After prolonged dark shipment, the plants experience a drastic change from total darkness to lighted conditions. The aim of this study was to investigate the effects of lower-temperature acclimation before shipping and light acclimation after dark storage on the quality and photosynthetic status of *Phalaenopsis* plants. Temperature acclimation at 25/20 °C day/night temperatures for 10 d before shipment reduced the severity of chilling injury in mature *Phalaenopsis* plants in a cool and dark environment. The increased tolerance to chilling injury after temperature acclimation was not attributed to the changes of abscisic acid concentration in leaves. In another experiment, sudden exposure to a photosynthetic photon flux (*PPF*) of 399 μmol·m^{-2}·s^{-1} after dark storage resulted in foliar chlorophyll degradation, restricted recovery of net CO_2 uptake rate, and severely reduced maximal quantum efficiency of the leaves. Based on these three parameters investigated, an acclimation light intensity of 140 μmol·m^{-2}·s^{-1} resulted in the best post-shipping

performance of the plants. A gradual increase from low to moderate light levels is suggested as the ideal light acclimation protocol for cultivation of mature *Phalaenopsis* plants after prolonged dark storage.

Additional key words: simulated dark shipping, chilling injury, abscisic acid, shipping temperature, net CO_2 uptake, chlorophyll fluorescence

Introduction

Phalaenopsis is an important floriculture crop. Production of potted *Phalaenopsis* is a growing industry, and it is common for plants in various stages of maturity to be exported for finishing and resale in other countries. Growers in warm climates mass-produce *Phalaenopsis* plants at high temperatures (e.g. 28-32 °C day temperatures among Taiwanese growers) to promote rapid growth during the vegetative period. Large quantities of mature plants ready for flower forcing with low temperature are exported to growers in cooler climates. *Phalaenopsis* plants exhibit crassulacean acid metabolism (CAM), hence they can tolerate shipping conditions well. The recent trend is for ready-to-force plants to be shipped by sea to save costs. Via the sea route, plants are typically shipped in total darkness at 18-20 °C for prolonged periods. For instance, it takes up to 3 weeks for *Phalaenopsis* plants from Taiwan to reach America or Europe by sea. Before and after the long-term dark shipping, the *Phalaenopsis* plants undergo at least two drastic changes, namely a drop in temperature by more than 10 °C as they are moved from the greenhouse to the shipping container, and a sharp increase in light level from total darkness during shipping to about 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ commonly used in the finishing stage of *Phalaenopsis* cultivation.

Exposure to low temperatures results in chilling injury in *Phalaenopsis*

(McConnell and Sheehan, 1978). Chilling injury symptoms in *Phalaenopsis* include water-soaked spots on leaf surfaces, yellowing of the leaves, and leaf drop. These symptoms are sometimes observed in *Phalaenopsis* plants after shipment. The protective effect of lower-temperature acclimation against chilling injury has been reported for *Phalaenopsis* plants in simulated 10-d air-freight conditions (Wang, 2007), but no detailed investigation of physiological parameters associated with chilling injury in *Phalaenopsis* plants has been reported.

A sharp increase in light level from prolonged period of total darkness to general greenhouse light levels may cause yellowing of leaves or sunburn. The lower leaves of *Phalaenopsis* are adapted to low light due to shading by upper leaves, but possess the ability to reacclimate to bright light (Lin and Hsu, 2004). The effect of light intensity on the performance of *Phalaenopsis* plants after dark storage has not been studied.

In this study, we subjected *Phalaenopsis* plants to a simulated 21-d dark shipping at different temperatures to investigate how temperature acclimation before shipping affects plant quality and investigate the relationship between different shipping temperatures and abscisic acid (ABA) concentration. In a separate experiment, the effect of various light intensities on the subsequent performance of plants after dark storage was investigated, in order to determine the optimal light intensity for *Phalaenopsis* plants just removed from storage in total darkness.

Materials and methods

Plant materials

All the experiments in this study used mature, ready-to-force *Phalaenopsis* Sogo Yukidian ‘V3’ purchased from Clone International Biotech (Pingtung, Taiwan). The

plants had 35- to 40-cm leaf span and were potted in 10.5-cm diameter, clear, soft plastic pots (0.75 L capacity) with tightly packed sphagnum moss medium. Plants were fertigated as necessary with a 20N-8.6P-16.6K fertilizer (Peters Professional 20-20-20; Scotts, Marysville, OH) at 1 $g \cdot L^{-1}$. Average day/night temperatures of 28/25 °C and a maximum photosynthetic photon flux (*PPF*) of 370 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were maintained in the greenhouse.

Simulated dark shipping conditions

Fertigation was discontinued approximately 7 days before simulated dark shipping (SDS) to reduce the risk of disease occurrence. Plants designated to receive SDS (hereafter referred to as "shipped plants") were put into cartons with shredded newspapers, and then placed in a dark growth chamber for 21 d. Unless stated otherwise, temperature was maintained at 20 °C. Relative humidity was 40% to 50% within the cartons during storage, as measured by a datalogger (H08-004-02; Onset, Buzzards Bay, MA).

Measurement of chlorophyll fluorescence and net CO_2 uptake rate

Measurements were taken on the newly matured leaf (second leaf from the apex), at the middle area near the midrib.

Leaf chlorophyll fluorescence was measured at 1300 HR with a photosynthesis yield analyzer (MINIPAM; Heinz Walz, Effeltrich, Germany). Minimal fluorescence (Fo) was determined after a 40-min dark adaptation. Maximal fluorescence (Fm) was measured after a saturation pulse. The maximal quantum efficiency Fv/Fm was calculated, where Fv = Fm – Fo.

A portable photosynthesis system (LI-6400; LI-COR, Lincoln, NE) was used to measure net CO_2 uptake rate. Because *Phalaenopsis* are CAM plants, the

measurements were done in the dark at 2100 HR in the greenhouse. Each measurement was taken on 6 cm^2 of leaf area. External air was scrubbed of CO_2 and then mixed with a supply of pure CO_2 to create a standard concentration of 350 $\mu mol \cdot mol^{-1}$. Air flow rate was controlled at 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$. During measurement, the leaf was kept in the leaf chamber for 20 to 30 s for equilibration with the ambient microconditions. Net CO_2 uptake rate and stomatal conductance (g_S) were recorded.

Measurement of ABA concentration

Leaf discs (≈0.2 g) were collected from the second leaves from the apex. The sampling point was at the middle portion of the leaf near the midrib. The sample was frozen in liquid N_2 after fresh weight measurement. Each sample was extracted with 200 μL of 80% methanol at 25 °C for 6 h. A 100 μL aliquot of each extract was put in a 96-well plate and dried at 45 °C. The samples were redissolved and separated with reverse-phase chromatography on columns packed with C_{18}-silica material (J.T. Baker Chemicals, Philipsburg, NJ). Indirect enzyme-linked immunosorbent assay (ELISA) as described by Setter et al. (2001) was used to analyze ABA.

Measurement of leaf color and relative chlorophyll concentration

Leaf color was measured with a spectrophotometer (CM-2600d; Konica Minolta Sensing, Osaka, Japan), at the same leaf position described for chlorophyll fluorescence measurement. Leaf relative chlorophyll concentration was measured at the tip of the newly matured leaf by a chlorophyll meter (SPAD502; Minolta, Tokyo, Japan).

Expt. 1. Effects of lower-temperature acclimation before shipping and the temperature during shipment on the subsequent quality and performance of *Phalaenopsis*

Plants were grown at 30/25 °C day/night temperatures for 10 days after purchase

to mimic the growing conditions in commercial greenhouses. Plants were divided into 8 groups of 12 plants each (as shown in Table 1). Temperature acclimation was done by transferring the plants to a 25/20 °C phytotron 10 days before SDS, while non-acclimated plants were kept at 30/25 °C during that period. Both temperature-acclimated and non-acclimated groups were subjected to SDS at 25, 20, and 15 °C. At the end of SDS, the number of yellowed leaves per plant was counted. A leaf with obvious yellow coloration, or with yellowing at the base, was defined as a "yellowed leaf". The degree of chilling injury of the plants was determined using the index from 0 to 3 as described in Table 1. The concentration of ABA in newly matured leaves was measured. Chlorophyll fluorescence parameters of newly matured leaves were determined at Day 0 and Day 3 after SDS ended. The control groups were not subjected to SDS treatment and the number of yellowed leaves, the degree of chilling injury, and the leaf ABA concentration of the plants were measured at the start of the SDS period. At the end of SDS, the plants were grown at 25/20 °C day/night temperatures, and the time to spiking (i.e. inflorescence emergence) was recorded.

Expt. 2. Determination of the optimal light intensity for acclimation after a prolonged dark storage

A total of 35 plants were subjected to SDS. The plants were then placed in a growth chamber with 30/25 °C day/night temperatures and given 34, 72, 140, 200, or 399 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF* at a 12-h photoperiod. The light source was high-pressure sodium lamps, and the various *PPFs* were achieved by applying shade cloths and adjusting the height of benches. Control plants were not subjected to SDS and were grown in a growth chamber at 30/25 °C with a *PPF* of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Net CO_2 uptake rate, g_S, and parameters of chlorophyll fluorescence were measured before storage and 0, 1, 2, 3, 4, 5, 9, 12, and 15 d after SDS was completed. Relative chlorophyll concentration and leaf color were determined 15 d after the end of storage.

After 15-day light acclimation, the plants were given 25/20 °C day/night temperatures for forcing at approximately 200-300 μmol·m^{-2}·s^{-1} *PPF*. The dates of spiking were then recorded.

Statistical analysis

Data were subjected to analysis of variance by using a completely randomized design. Means separation between treatments was determined with the least significant difference (LSD) test at $P \leq 0.05$. Statistical analyses were performed with Costat (version 6.1; CoHort Software, Monterey, CA).

Results

Expt. 1. Effects of lower-temperature acclimation before shipping and the temperature during shipment on the subsequent quality and performance of *Phalaenopsis*

Storage temperature during SDS affected the number of yellowed leaves. Plants stored at 25 °C had the highest number of yellowed leaves, while no difference in yellowing was found between plants stored at 20 and 15 °C (Table 1). There was no difference in number of yellowed leaves between acclimated and non-acclimated plants (Table 1). After storage at 15 °C, the plants suffered from chilling injury whether or not they had been acclimated, but less severe chilling injury was observed in plants with lower-temperature acclimation (Table 1). Storage temperature affected the ABA concentration in the leaves, with the highest ABA concentrations detected in plants stored at 25 °C; the ABA concentration at that temperature was similar to control plants not subjected to SDS (Table 1). Temperature acclimation of plants before storage did not affect the ABA concentration, indicating that the increased tolerance to chilling injury observed in temperature-acclimated plants shipped at 15 °C was not due to ABA accumulation (Table 1). The quality of the plants was

preserved best when stored at 20 °C.

Leaf Fv/Fm values of plants right after SDS were affected by storage temperature, where the leaves of plants stored at 25 °C had lower Fv/Fm values than the other storage temperature groups, indicating an inhibition of PS II system on plants after the dark storage at higher temperature (Table 2). Whether or not the plants had been acclimated, plants stored at 25 °C had slightly higher Fo but significantly lower Fm than the other groups, indicating that the decrease in Fv/Fm at 25 °C was caused by lower Fm values (Table 2). There was no difference in leaf chlorophyll fluorescence parameters of all plants 3 days after SDS was ended (Table 2), demonstrating that the inhibition of PS II in *Phalaenopsis* due to 21-d dark storage at 25 °C was temporary and reversible.

Temperature-acclimated plants required shorter forcing period after SDS before spiking; for example, after 21-d storage at 20 °C, acclimated plants required 18.0 days to spiking on average, compared with 24.5 days for non-acclimated plants (data not shown).

Expt. 2. Determination of the optimal light intensity for acclimation after a prolonged dark storage

The effect of different light levels during the recovery period after 21-d SDS was determined. Low net CO_2 uptake rate was observed in all shipped plants at the end of the SDS (Fig. 1A). One day after SDS was ended, various levels of *PPF* produced a small increase in net CO_2 uptake rate, and the values in the different treatments were similar. Under 140 and 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF*, net CO_2 uptake rate of the plants increased rapidly to the level of control plants within 4 d after SDS was ended (Fig. 1A). Under 34 and 72 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF*, net CO_2 uptake rate of the plants increased with time in the early recovery period, but soon tapered off after 4 to 5 d and did not

reach the level of control plants within 15 d after the end of SDS (Fig. 1A). The recovery of CO_2 uptake was greatly inhibited by a high light intensity (399 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and increased slowly with time during the recovery period; even after 15-d exposure to light, the net CO_2 uptake rate was just a little more than half the value measured in control plants (Fig. 1A). The g_S exhibited a similar trend to that of net CO_2 uptake by and large (Fig 1B). The relationship between *PPF* and net CO_2 uptake rate changed with time after SDS (Fig. 2). There was little correlation between *PPF* and net CO_2 uptake rates on Day 1 ($R^2 = 0.23$, $P = 0.02$) and the net CO_2 uptake rates were similarly low in all shipped plants regardless of light intensity given. On Day 2, net CO_2 uptake rate of shipped plants increased markedly except for those given 399 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF* (Fig. 2). Net CO_2 uptake rates and *PPF* were found to be quadratically correlated on Days 2 through 9, and the curve maxima shifted to the direction of increasing *PPF* with time after storage, indicating that there is a gradual increase over time in the light requirement of *Phalaenopsis* plants after a dark storage (Fig. 2). However, a *PPF* value of 399 $\mu mol \cdot m^{-2} \cdot s^{-1}$ always corresponded to a sharp decline of net CO_2 uptake on the curves (Fig. 2). These results showed that net CO_2 uptake of shipped plants was able to recover quickly to a normal level at a medium light intensity (140-200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF*), but was restricted at low (34-72 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF*) and high (399 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF*) light intensities.

Just after SDS was ended, the maximal quantum efficiency (Fv/Fm) of shipped plants were similar to that of control, indicating that PS II of plants was not damaged during storage (Table 3). After exposure to light, all treatments showed a decrease in Fv/Fm in varying degrees by Day 1 (Table 2), where the largest drop was found under 399 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF* and less significant on 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF* (Table 3). Lower light intensities resulted in very slight decrease in Fv/Fm, and the values were still comparable to that of control plants (Table 3). By Day 3 through 12, the Fv/Fm levels of shipped plants acclimated in lower light intensities (34-140 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF*)

became stabilized at values close to that of control plants, while the values were slightly and significantly lower, respectively, for plants under 200 and 399 μmol·m^{-2}·s^{-1} *PPF* (Table 3).

After 15-d light acclimation, the shipped plants showed decreased relative chlorophyll concentration as represented with SPAD values, and increased L, a, and b values as the light intensity given to the plants was increased (Fig. 3). Higher L, a, and b values represented lighter color, less green coloration, and more yellow coloration, respectively. The leaf relative chlorophyll concentration of plants given 399 μmol·m^{-2}·s^{-1} *PPF* was significantly lower than that of control plants, while that of plants given 200 μmol·m^{-2}·s^{-1} *PPF* were only moderately lower compared with control (Fig. 3A). The L, a, and b values of plants at 399 μmol·m^{-2}·s^{-1} *PPF* indicated significant lightening of green color and more pronounced yellow hue, while the leaf color parameters of plants given 140 μmol·m^{-2}·s^{-1} and 200 μmol·m^{-2}·s^{-1} *PPF* were comparable to control (Fig. 3B to D). Deeper green hue in the leaves was detected in plants given 34 and 72 μmol·m^{-2}·s^{-1} *PPF* (Fig. 3B to D).

Among the shipped plants, those acclimated at 140 μmol·m^{-2}·s^{-1} *PPF* produced the best spiking quality, i.e. similar timing and percentage of spiking compared with control plants, with 100% spiking achieved by Day 60 after forcing was started (data not shown). At the two lowest light intensities during acclimation, some early spiking was observed by Day 20, but lower spiking percentages was observed on Day 60 (data not shown). Acclimation at *PPFs* of 34, 72 and 399 μmol·m^{-2}·s^{-1} resulted in only 71% spiking by Day 60; at the same day, the observed the percentage of spiking at 200 μmol·m^{-2}·s^{-1} *PPF* was 86% (data not shown). Shipped plants acclimated at 399 μmol·m^{-2}·s^{-1} *PPF* had the most non-uniform spiking overall, with only half the spiking percentage of control at Day 40 (data not shown).

Table 1. Effects of thermal acclimation at 25/20 °C for 10 days, and temperature of simulated dark shipping on subsequent leaf condition, degree of chilling injury, and abscisic acid (ABA) concentration of *Phalaenopsis* Sogo Yukidian 'V3'. Treated plants were stored in a cooler for 21 days (n = 12).

Storage temp. (°C)	Temp. acclimation	Before simulated dark shipping			The end of simulated dark shipping		
		Yellowed leaf (no.)	Degree of chilling injury[y]	ABA concn. (pmol·g^{-1}FW)	Yellowed leaf (no.)	Degree of chilling injury	ABA concn. (pmol·g^{-1}FW)
None[z]	No	0.0	0.0	10.0			
None	Yes	0.0	0.0	9.6			
25	No				0.6	0.0	10.4
	Yes				0.6	0.0	10.4
20	No				0.1	0.1	9.8
	Yes				0.0	0.0	7.8
15	No				0.2	2.1	8.2
	Yes				0.1	1.3	7.2
Significance							
Storage temp.		-	-	-	**	***	*
Temp. acclimation		NS	NS	NS	NS	*	NS
Temp. acclimation X Storage temp.		-	-	-	NS	*	NS

[z] Data were collected on plants grown in greenhouse with day/night temperatures of 30/25 °C before the simulated dark shipping.
[y] 0 = no chilling injury; 1 = slight water-soaked spots on leaf surface, reversible after subsequent growth; 2 = one leaf had a yellowed leaf base; 3 = two or more leaves had obvious yellow spots, which covered more than 20% of the leaf areas.
NS,*,**,*** Nonsignificant or significant at $P \leqq 0.05$, 0.01, or 0.001, respectively.

Table 2. Effects of thermal acclimation at 25/20 °C for 10 days, and temperature of simulated dark shipping on subsequent chlorophyll fluorescence parameters of *Phalaenopsis* Sogo Yukidian 'V3'. Treated plants were stored in a cooler for 21 days (n = 6).

Storage temp. (°C)	Temp. acclimation	Before simulated dark shipping			The end of simulated dark shipping			3 days after the end of simulated dark shipping		
		Fv/Fm	Fo	Fm	Fv/Fm	Fo	Fm	Fv/Fm	Fo	Fm
None[z]	No	0.82	352	2008						
None	Yes	0.83	352	2026						
25	No				0.77	314	1346	0.78	328	1495
	Yes				0.74	325	1256	0.79	321	1533
20	No				0.79	298	1432	0.77	334	1480
	Yes				0.80	302	1544	0.77	335	1472
15	No				0.80	308	1564	0.78	331	1518
	Yes				0.79	322	1540	0.78	321	1488
Significance										
Storage temp.		-	-	-	***	*	***	NS	NS	NS
Temp. acclimation		NS	NS	NS	NS	NS	NS	NS	NS	NS
Temp. acclimation X Storage temp.		-	-	-	NS	NS	*	NS	NS	NS

[z] Data were collected on plants grown in a greenhouse with day/night temperatures of 30/25 °C before the simulated dark shipping.
NS,*,*** Nonsignificant or significant at $P \leqq 0.05$, or 0.001, respectively.

Table 3. Changes of maximal quantum efficiency (Fv/Fm) in *Phalaenopsis* Sogo Yukidian 'V3' 0, 1, 3, 5, 9, and 12 d after a 21-d simulated dark shipping at 20 °C. Various levels of photosynthetic photon flux (*PPF*) as indicated were provided after the simulated dark shipping. (Hou and Chang, 2010)

PPF ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Time after the end of simulated dark shipping (d)					
	Day 0	Day 1	Day 3	Day 5	Day 9	Day 12
Control[z]	0.79 b[y]A[x]	0.79 a A	0.79 a A	0.78 ab A	0.78 ab A	0.77 a A
399	0.81 a A	0.62 c B	0.39 c C	0.41 c C	0.31 c C	0.38 b C
200	0.81 a A	0.71 b B	0.72 b B	0.72 b B	0.73 b B	0.74 a B
140	0.81 a A	0.76 a C	0.77 ab BC	0.77 ab BC	0.79 ab B	0.78 a B
72	0.82 a A	0.79 a B	0.81 a A	0.82 a A	0.82 a A	0.82 a A
34	0.81 a AB	0.80 a B	0.81 a A	0.82 a A	0.81 a A	0.82 a A

[z] Plants were not subjected to simulated dark shipping and grown in a growth chamber with day/night temperature of 30/25 °C and with a *PPF* of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

[y] Means followed by a different lower case letter in columns are significantly different at $P \leq 0.05$ by the least significant difference (LSD) test (n=7).

[x] Means followed by a different upper case letter in rows are significantly different at $P \leq 0.05$ by the LSD test.

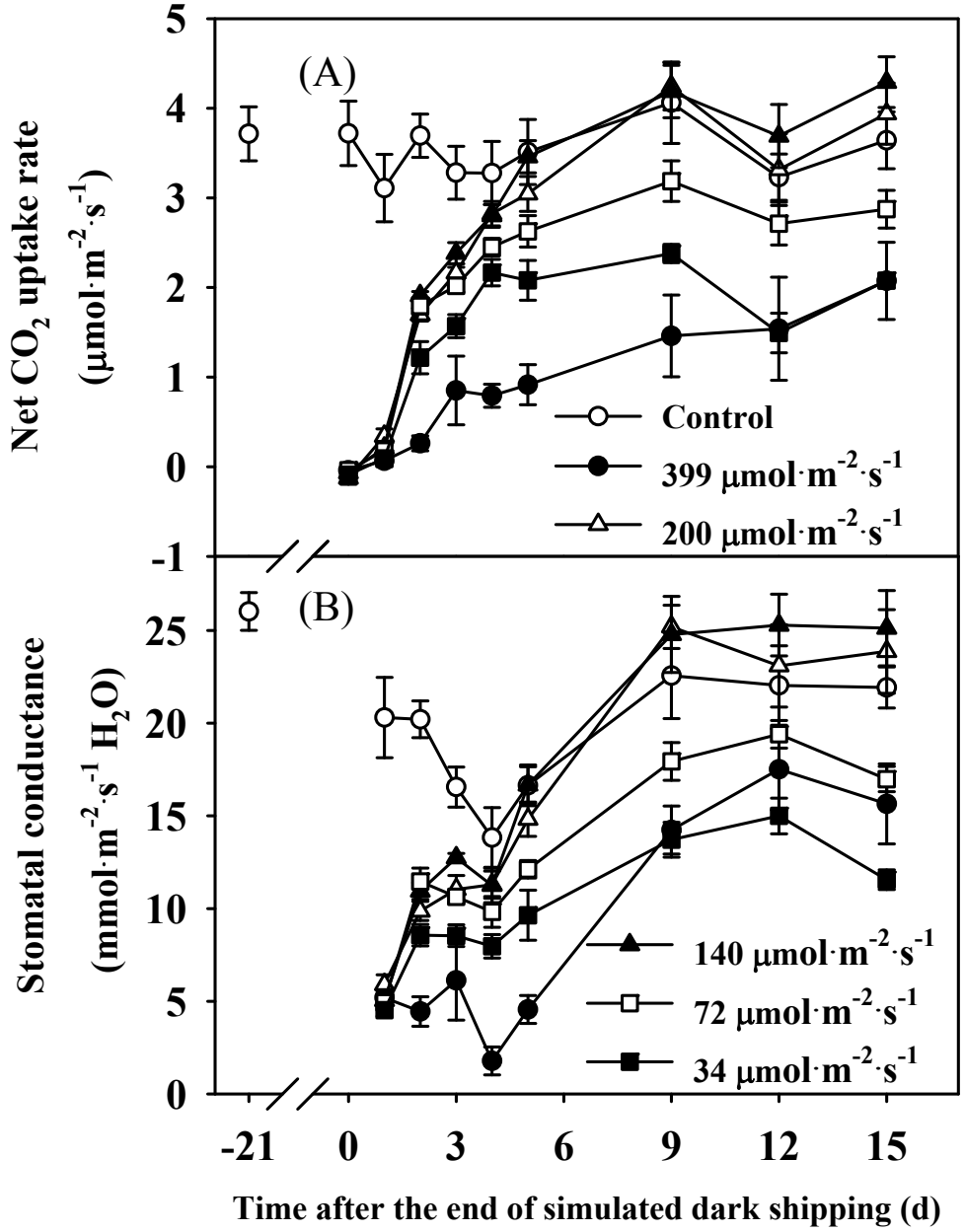


Fig. 1. Effect of light acclimation at various levels of photosynthetic photon flux (*PPF*) on net CO_2 uptake rate (A) and stomatal conductance (B) in *Phalaenopsis* Sogo Yukidian 'V3'. Measurements were taken before and 0, 1, 2, 3, 4, 5, 9, 12, and 15 d after a 21-d simulated dark shipping at 20 °C. Controls did not receive simulated shipping and were grown in a growth chamber with day/night temperatures of 30/25 °C and with a *PPF* of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. All shipped plants were moved to the same growth chamber as controls but received various *PPF* as indicated after the simulated dark shipping. Bars indicate SE of the means (n=7). (Hou et al., 2010)

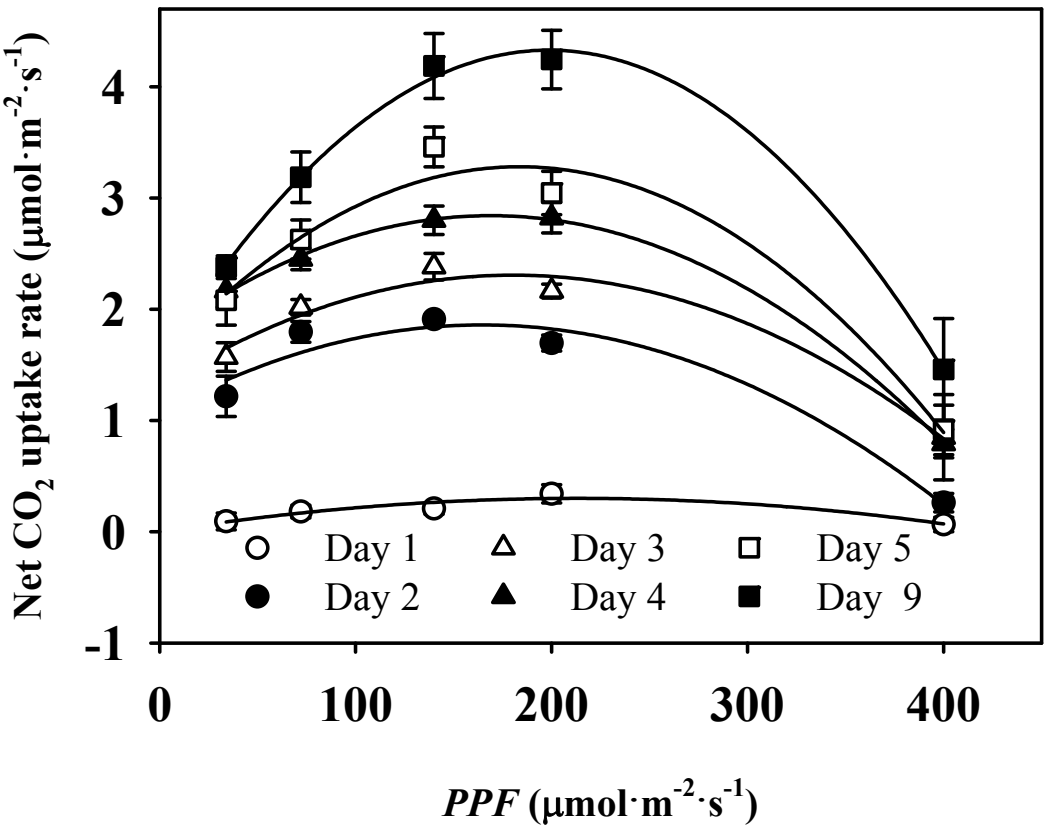


Fig. 2. The relationship between photosynthetic photon flux (*PPF*) and net CO_2 uptake rate of *Phalaenopsis* Sogo Yukidian 'V3' on Day 1 (-○-, y=0.0002 + 0.0028x-0.0000066x^2, R^2=0.23, *P*=0.02), Day 2 (-●-, y=1.07+0.0096x-0.0000292x^2, R^2=0.81, *P*<0.0001), Day 3(-△-, y=1.31+0.011x-0.0000305x^2, R^2=0.55, *P*<0.0001), Day 4 (-▲-, y=1.75 + 0.013x-0.0000.83x^2, R^2=0.85, *P*<0.0001), Day 5 (-□-, y=1.56 + 0.019x-0.000051x^2, R^2=0.74, *P*<0.0001), and Day 9 (-■-, y=1.52 +0.028 x -0.000071x^2, R^2=0.69, *P*<0.0001) from the completion of simulated dark shipping. Bars indicate SE of the means (n=7). (Hou et al., 2010)

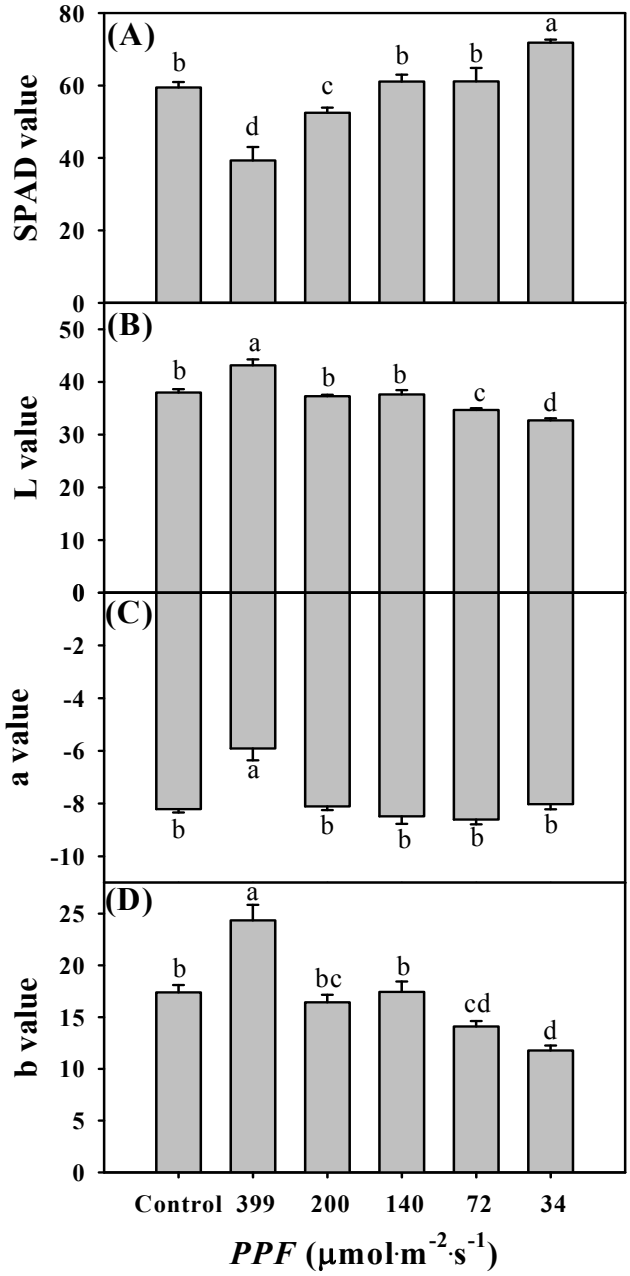


Fig. 3. Effect of 15-d light acclimation with various photosynthetic photon flux (*PPF*) on relative leaf chlorophyll concentration and leaf color of *Phalaenopsis* Sogo Yukidian 'V3' after a 21-d simulated dark shipping, as measured by SPAD and Lab values, respectively. Controls were not subjected to simulated shipping and were grown in a growth chamber with day/night temperatures of 30/25 °C and with a *PPF* of 200 μmol·m^{-2}·s^{-1}. Shipped plants were placed in the same growth chamber as controls but received various *PPF*s as indicated after the simulated dark shipping. (Hou and Chang, 2010)

Discussion

The shipping temperature of *Phalaenopsis* plants by sea route is set between 18-20 °C on average. A storage temperature of 20 °C was found to preserve the quality of the plants well during simulated shipment, whereby none to very slight chilling injury was observed on both temperature-acclimated and non-acclimated plants (Table 1). However, incidence of chilling injury was significant at 15 °C, and plants acclimated to lower temperature before shipment could better withstand the effects of chilling (Table 1). Therefore, temperature acclimation of *Phalaenopsis* plants before shipment is highly recommended, because different clones may have different susceptibility to chilling injury, and plants may be subjected to greater temperature drop in practice if they were grown in warmer greenhouses. On the other hand, we found that a shipping temperature of 25 °C had detrimental effects on the shipped plants (Tables 1 and 2).

Prolonged dark shipping at 20 °C did not affect PS II in *Phalaenopsis* plants as indicated by Fv/Fm value comparable to the level found in control plants (Tables 2 and 3). However, different light intensities during the recovery period after shipping were found to significantly affect net CO_2 uptake rate, Fv/Fm, and relative chlorophyll concentration of newly matured leaves. After 21 d of total darkness, a sudden exposure to high light levels commonly used for growing *Phalaenopsis* in the finishing stage (399 $\mu mol \cdot m^{-2} \cdot s^{-1}$) produced symptoms of sunburn, and decreased the Fv/Fm and net CO_2 uptake rate of the leaves (Table 3, Figs. 1-3). Therefore, acclimation at low to moderate light levels is necessary to enhance the performance of shipped plants. Effects of low to moderate light levels during the acclimation period were compared, and continuous low light levels did not damage the plants but resulted in restricted net CO_2 uptake. Shipped plants given moderate light levels of 140 and 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF* recovered the uptake of CO_2 quickly to normal levels (Fig. 1),

but a direct exposure to 200 μmol·m^{-2}·s^{-1} *PPF* still caused slight damaging effects on the leaves (Table 3, Fig. 3). Further analysis of the data showed that the light requirement of *Phalaenopsis* plants after prolonged dark shipping gradually increased with time (Fig. 2). Hence a gradual increase from low to moderate light level is recommended for 6 to 9 d after prolonged dark shipping. However, if gradual adjustment of light levels over a few days is not feasible, a direct acclimation at 140 μmol·m^{-2}·s^{-1} *PPF* is acceptable.

Acknowledgements

We offer our thanks to Dr. Tim L. Setter for performing the ABA analysis. We are very grateful for the financial support provided by the Council of Agriculture, Executive Yuan, Taiwan [96AS-1.2.1-ST-a1(4) and 97AS-1.2.1-ST-a1(3)].

Literature cited

Hou, J. Y. and Y. C. A. Chang. 2010. Effect of light acclimation on photosynthetic status and post-shipping performance after a long-term dark storage in *Phalaenopsis* Sogo Yukidian 'V3'. The Proceedings of the 28th International Horticultural Congress. 22-27 August, 2010. Lisbon.

Hou, J. Y., T. L. Setter, and Y. C. A. Chang. 2010. Effects of simulated dark shipping on photosynthetic status and post-shipping performance in *Phalaenopsis* Sogo Yukidian 'V3'. J. Amer. Soc. Hort. Sci. 135: 183-190.

Lin, M. J. and B. D. Hsu. 2004. Photosynthetic plasticity of *Phalaenopsis* in response to different light environments. J. Plant Physiol. 161: 1259-1268.

McConnell, D. B. and T. J. Sheehan. 1978. Anatomical aspects of chilling injury to

leaves of *Phalaenopsis* Bl. HortScience 13: 705-706.

Setter, T. L., B. A. Flannigan, and J. Melkonian. 2001. Loss of kernel set due to water deficit and shade in maize: Carbohydrate supplies, abscisic acid, and cytokinins. Crop Sci. 41: 1530-1540.

Wang, Y. T. 2007. Temperature, duration in simulated shipping, and thermal acclimatization on the development of chilling injury and subsequent flowering of *Phalaenopsis*. J. Amer. Soc. Hort. Sci. 132: 202-207.

國家圖書館出版品預行編目資料

台美觀賞作物栽培技術與分子輔助育種研討會專刊. 2010= Proceedings of the Taiwan -USA Symposium on Technology of Cultivation and Molecular Breeding for Ornamental Crops / 謝廷芳等主編.－初版.－臺中縣霧峰鄉：農委會農試所, 民 99.08
面: 21*29.7 公分.

ISBN (平裝) 978-986-02-4616-2

1. 花卉 2. 栽培 3. 分子育種 4. 文集

2010 台美觀賞作物栽培技術與分子輔助育種研討會專刊

編　　輯：謝廷芳、戴廷恩、汪澤宏
出 版 者：行政院農業委員會農業試驗所
地　址：台中縣霧峰鄉中正路 189 號
網　址：http://www.tari.gov.tw
印 製 者：農世股份有限公司
定　　價：平裝 新台幣 350 元
出版日期：中華民國九十九年八月　初版
展 售 處：國家書店松江門市
台北市松江路三段 209 號 TEL:02-25180207
五南文化廣場
台中市中山路 2 號 TEL:04-22260330
國家網路書店：http://www.govbooks.com.tw
ISBN：978-986-02-4616-2（平裝）
GPN：1009902716

—版權所有　翻印必究—